JOURNAL OF AGRICULTURAL RESEARCH

VOL. 36 WASHINGTON, D. C., APRIL 1, 1928 No. 7

INHERITANCE OF EARLINESS AND OTHER AGRONOMIC CHARACTERS IN RICE [1]

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INTRODUCTION

Rice growing in California on a commercial scale began in 1912. The leading commercial variety grown during the first five years was Wataribune, a late-maturing, short-grain rice. This variety later was replaced by early and midseason short-grain varieties. Some of these varieties were introductions from Japan, while others were pure-line selections distributed from the Biggs Rice Field Station.

In California, early and midseason rices, especially the early-maturing varieties, do not yield as well, as a rule, as the late-maturing varieties. However, the danger of loss of crop due to wet weather in the fall largely is eliminated by the use of early-maturing rices. For this reason there is a constant demand for early-maturing rice varieties that will yield and mill well. Many early varieties are available, but those which have been tested in California do not yield as well, except on rich soil, as do the midseason and late-maturing varieties.

There have not been reported, so far as can be found, any studies in the United States on inheritance in rice, though the crop has been grown in this country since 1694.

The work reported in this paper began in 1922. A cross was made by the writer in that year, using an early-maturing variety, Niro Vialone, as the female parent, and a midseason variety, Caloro, as the male parent. The cross was made to determine whether by recombination of characters in succeeding generations it would be possible to secure a higher-yielding early-maturing variety than those which were available. A study of the inheritance of several contrasted characters other than earliness was made in the F_2 and F_3 generations.

MATERIAL AND METHODS

The most accurate index of earliness in rice is the appearance of the upper spikelets of the panicle above the leaf sheath. This index of earliness was found to be more satisfactory than a record of the date when the plant is fully headed or the kernels are all ripe. "Fully headed," as used in this study, means that all panicles on a plant were

[1] Received for publication Dec. 15, 1927; issued June, 1928. Experiments conducted in cooperation with the California Agricultural Experiment Station.

[2] The writer desires to thank R. E. Clausen, of the genetics department of the University of California, for his suggestions, and C. F. Dunshee and W. D. Comstock for the taking of notes on the F_3 population during his absence from the station in 1925.

exserted from the sheaths. "All ripe" means that the kernels on the lower part of the panicle were in the stiff-dough stage, the kernels of the upper spikelets were hard, and the lemmas were straw colored.

Niro Vialone, the female parent, is an early-maturing variety with many organs purplish in color. The average date of maturity for this variety during the six-year period from 1918 to 1923, inclusive, was September 14. It normally is awnless, but occasionally a purplish awn, varying in length from 1 to 2 mm., may be observed. The lemma tips, the glumes, the stigmas, the pulvinuses, the bases of the ligules, the auricles, the leaf sheaths, the leaf blades, the nodes, and the internodes are purplish in color. The upper portion of the peduncle or internode which supports the panicle often is called the "neck." In Niro Vialone the neck is sinuous instead of straight, as in most varieties.

Caloro, the male parent, is a midseason normally green variety. The average date of maturity for this variety during the six-year period from 1918 to 1923, inclusive, was October 4. Normally about one-half the spikelets on a panicle are awnless, but the remaining spikelets of Caloro have white awns which are variable in length. The plant organs which are described as colored in the Niro Vialone variety are green or uncolored in Caloro. The peduncle or neck is straight.

INHERITANCE OF EARLINESS

In making the cross all but about 20 spikelets were clipped from the panicle of the female parent plant. The remaining spikelets were emasculated one morning and pollinated on the following day with pollen collected at random from plants of the male parent. Two crossed seeds were obtained.

F_1 PROGENY

The two crossed seeds were sown in 1923, but unfortunately one failed to germinate. The F_1 plant secured was bagged during the blossoming period to avoid the possibility of cross-fertilization. In date of maturity the F_1 plant was intermediate, but nearer the early parent.

It may be of interest to include here data on the dates of first spikelet emergence of F_1 hybrids from other rice crosses that were grown at Biggs in 1925.

On May 13, 1925, F_1 seed from nine crosses and seed of all parent varieties were sown in paper pots, one seed in each pot. After the seedlings had emerged the bottoms of the pots were removed and the seedlings were transplanted in rod rows spaced 2 feet apart. The seedlings were spaced about 1 foot apart in the rows. The plants, therefore, had ample room for development.

Notes were taken on the date of first spikelet emergence for each individual F_1 and parent plant. First spikelet emergence, as used in this paper, means that two or more spikelets of the earliest panicle were showing above the leaf sheaths. In Table 1 are presented the data on the average date of first spikelet emergence for the nine crosses and the parent varieties.

TABLE 1.—*Average date of first emergence of spikelets of the parent varieties and the F_1 plants from nine rice crosses grown at Biggs, Calif., in 1925*

Female parent			Male parent			F_1 progeny	
Name	Number of plants	Average date of emergence of first spikelets	Name	Number of plants	Average date of emergence of first spikelets	Number of plants	Average date of emergence of first spikelets
C. I. No. 5346	5	Aug. 15	Italian red	6	Aug. 10	7	Aug. 9
Niro Vialone	7	Aug. 5	Wataribune	5	Sept. 4	2	Aug. 29
Caloro	6	Aug. 28	Viola	7	Aug. 15	7	Aug. 27
Colusa	5	Aug. 13	Italian red	6	Aug. 10	3	Aug. 18
Do	5	do	Lady Wright	7	Aug. 22	29	Aug. 28
Lady Wright	7	Aug. 22	Caloro	6	Aug. 28	30	Aug. 29
Caloro	6	Aug. 28	Lady Wright	7	Aug. 22	23	Aug. 28
Wataribune	5	Sept. 4	Red rice		Aug. 23[a]	6	Oct. 16
Caloro	6	Aug. 28	C. I. No. 5315	5	Aug. 21	33	Oct. 19

[a] In 1926.

For the cross C. I. (Cereal Investigations) No. 5346 × Italian red, the F_1 plants began to head earlier than those of either parent. For the crosses Niro Vialone × Wataribune and Caloro × Viola the F_1 plants were nearer the late parent in this character. For the cross Lady Wright × Caloro and its reciprocal, the F_1 plants were about the same as the late parent. For the crosses Colusa × Italian red and Colusa × Lady Wright, the F_1 plants were considerably later than the late parent. For the crosses Wataribune × red rice and Caloro × C. I. No. 5315, the F_1 plants were 42 and 52 days later, respectively, than the late parent in first emergence of spikelets. No mature seed was produced by F_1 plants of either of these two crosses. Yet, in rows adjacent to the F_1 plants Wataribune matured on October 18 and red rice on October 4 (1926), Caloro on October 9, and C. I. No. 5315 on October 5.

Both red rice and C. I. No. 5315 have wider leaves, tiller more freely, and shatter much more readily than do the Caloro and Wataribune. These two first-named varieties also have a spreading habit of growth and rather open panicles, whereas Caloro and Wataribune are erect in growth and have panicles of average compactness. This unexpected behavior of the F_1 plants in these two crosses suggests that the red rice and C. I. No. 5315 varieties may belong to a different species from Caloro and Wataribune, which are Japanese varieties.

The results presented here indicate that in rice F_1 plants from various crosses may be earlier than the early parent, later than the late parent, or nearer the early or the late parent in time of first emergence of spikelets. The parents also may be so different that the F_1 plants are sterile.

F_2 PROGENY

Hoshino (*5*)[3] found in a cross between early and late-maturing rice that the time of flowering in F_1 was intermediate but nearer that of the early parent. In F_2 segregation was complex, but no transgressive inheritance occurred. In F_3 some families showed results very much like those from the parents. The author believed that the inheritance of earliness in this case could be explained on a three-

[3] Reference is made by number (italic) to "Literature cited," p. 601.

factor hypothesis. Ikeno (*6*) reports that, in crosses of early and late rices, the F_1 was intermediate and segregation in F_2 was complex and apparently due to multiple factors. Hector, according to Evans (*4*), found that the F_2 progeny from a cross between a colored autumn (early) rice and an uncolored winter (late) rice segregated into two distinct groups with respect to date of flowering. These two flowering periods were nearly the same as the flowering dates of the two parents, with an interval of about three weeks during which time no blooming occurred. The ratio of early to late plants was approximately 1 : 3. Bhide (*2*) found that lateness was dominant over earliness in two varieties with which he worked, causing ratios for the cross and its reciprocal of approximately 2.84 and 2.75 late plants to 1 early, respectively. But in other crosses the dominance of lateness over earliness was not very evident.

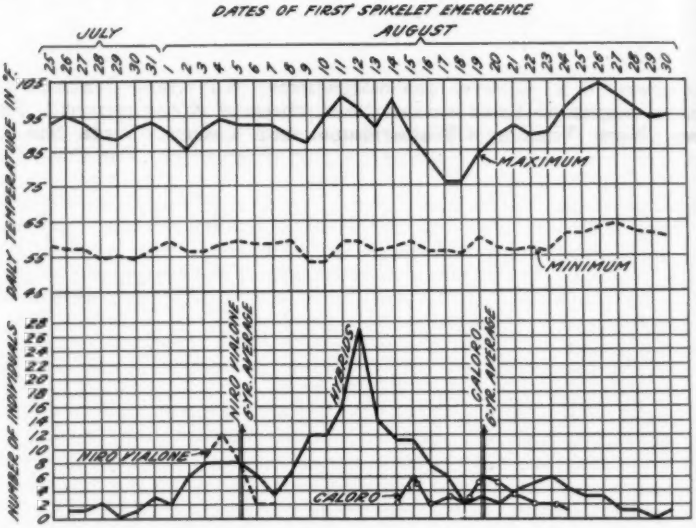

FIG. 1.—Frequency polygon showing dates of first spikelet emergence for the plants of the F_2 progeny and parent varieties of the Niro Vialone×Caloro rice cross grown at Biggs, Calif., with the daily maximum and minimum temperatures during the period

Nomura and Yamazaki (*7*) found that the F_1 hybrids were a few days later in shooting than was the late parent, and in F_2 segregation was in the ratio of about 3 late to 1 early plant. The late plants on an average were slightly later and the early plants slightly earlier than the late and early parents, respectively. The F_3 progeny from the F_2 early type showed the monomodal narrow variation. The F_2 late type produced two groups of plants, one showing the narrow monomodal variation, while the other segregated again into about 3 late to 1 early plant. The authors reached the conclusion that, with respect to factorial composition, the rices studied were as follows:

Cross No. 1 AAbbcc×aabbCC (early×late), cross No. 2 aabbCC×aaBBcc (late×early). When all these factors are present the shooting time was found to be earlier, 91 (1921, 1922) or 100 days (1923). Each of the factors A, B, and C prolongs the shooting time to a certain extent, C being most and B least

efficacious in this respect. By the combination of either A and C or B and C the shooting time becomes longer.

On May 9, 1924, the seeds obtained from the F_1 plant of the cross Niro Vialone × Caloro was sown on river-bottom soil in tubs. The F_2 plants emerged on May 18 and, when about 6 inches high, were transplanted to the nursery in rows 17 feet long and 3 feet apart. The plants were spaced about 6 inches apart in the rows. The parent material, grown for comparative purposes, likewise was sown in tubs and transplanted at the same time as the F_2 plants.

The index of earliness used in this study was the time of appearance of the first spikelet above the leaf sheath. This datum can be more accurately taken than others that might be used in a study of this nature with rice. The date of spikelet emergence, as used in this paper, therefore, means the appearance of the first spikelet above the leaf sheath.

In the cross Niro Vialone × Caloro the F_1 plant was intermediate in date of spikelet emergence, though slightly nearer the early parent. The frequency distribution of the plants of the Niro Vialone and Caloro parents and of the F_2 progeny is shown in Table 2, and a frequency polygon is given in Figure 1.

TABLE 2.—*Frequency distribution of the parents and F_2 progeny of the Niro Vialone × Caloro rice cross by dates of first spikelet emergence of plants at Biggs, Calif., in 1924*

Date of emergence of first spikelet	Parents and progeny (number)			Date of emergence of first spikelet	Parents and progeny (number)		
	Niro Vialone	Caloro	F_2 progeny		Niro Vialone	Caloro	F_2 progeny
July 26			1	Aug. 14		2	11
July 27			1	Aug. 15		6	11
July 28			2	Aug. 16		2	7
July 29			0	Aug. 17		3	6
July 30			1	Aug. 18		2	2
July 31			3	Aug. 19		6	3
Aug. 1			2	Aug. 20		5	2
Aug. 2			6	Aug. 21		3	4
Aug. 3	9		8	Aug. 22		2	5
Aug. 4	12		8	Aug. 23		2	6
Aug. 5	7		8	Aug. 24		1	4
Aug. 6	2		6	Aug. 25			3
Aug. 7	2		3	Aug. 26			2
Aug. 8			7	Aug. 27			1
Aug. 9			12	Aug. 28			1
Aug. 10			12	Aug. 29			0
Aug. 11			16	Aug. 30			1
Aug. 12			27				
Aug. 13			14	Total	32	34	203

Inspection of Table 2 shows that a number of F_2 plants began to exsert the upper spikelets earlier than the earliest of the early-parent plants, and likewise a number of F_2 plants began to exsert the uppermost spikelets later than the latest of the late-parent plants. This shows transgressive inheritance.

The frequency polygon shown in Figure 1 is trimodal, with a small early group, a large intermediate group, and a small midseason group of plants. The earliest F_2 plant began to exsert flowers eight days earlier than the earliest early-parent plant, and the latest F_2 plant began to flower six days later than the latest late-parent plant. It is possible that the lowering of the maximum temperature from

August 16 to August 19, inclusive, may have caused the low frequency of flowering on these dates. Akemine (*1*) found that rice flowers will open at a minimum temperature of 59° F. He also found, however, that the maximum of opening is at temperatures varying from 95° to 104° F., and that the higher the natural temperature the more active is the process of blooming.

If it is assumed that the depression in flowering observed from August 16 to August 19 was due to the lowering of the maximum daily temperature at this time, which is not improbable, the F_2 progeny would then be distributed into two groups, an early and a midseason one extending well beyond the midseason parent in time of spikelet exsertion. By using August 7, the date by which all the early-parent plants had begun to exsert spikelets, as the limit classes of the two and assigning two of the plants which began to flower on this date to the early group and one to the midseason group, there then would be 48 plants in the early group and 159 plants in

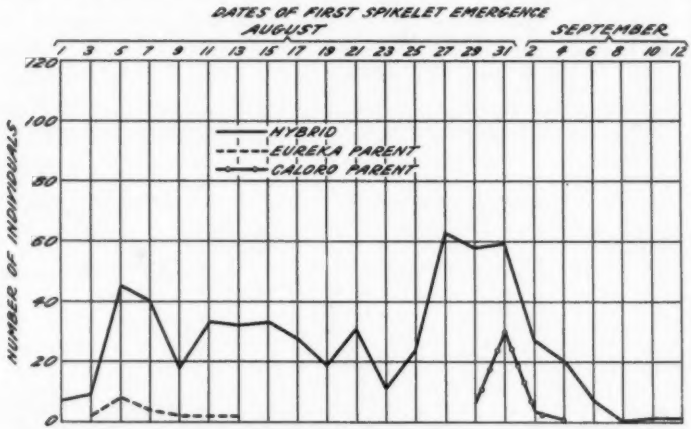

FIG. 2.—Frequency polygon showing dates of first spikelet emergence for the plants of the F_2 progeny and parent varieties of the Eureka×Caloro rice cross grown at Biggs, Calif., in 1925

the midseason group. This is approximately 3 midseason plants to 1 early, which suggests a single-factor difference for earliness. With this grouping the deviation from the 3:1 ratio is 3.75 ± 4.20, and is not significant.

Again, if the F_2 population is divided into early and midseason groups by using the modal frequency class (August 12) as the dividing line and assigning one-half of the plants in this modal class to each group, there are 109 plants in the early group and 98 plants in the late one. This indicates a 1:2:1 ratio with most of the F_2 plants intermediate in blooming time between those of the parents.

By using August 7 and August 18 as group limits, and distributing individuals flowering on August 7 as indicated in the second preceding paragraph, which gives 48 early, 126 intermediate, and 33 late plants, there are 23.2 per cent of the plants in the early group, 60.9 per cent in the intermediate group, and 15.9 per cent in the late group. These percentages indicate that, with respect to earliness, the F_2 progeny at least suggests a 1:2:1 ratio of early, inter-

mediate, and midseason groups, respectively. These ratios, however, are only suggestive, for the transgressive inheritance in this case indicates that more than a single-factor difference is involved in the production of earliness.

The date of first spikelet emergence also was studied in F_2 plants from the cross Eureka × Calero, grown in 1925. Eureka is an early-maturing variety. The F_1 in this cross was intermediate, but nearer the late parent. In F_2, transgressive segregation for date of first spikelet emergence occurred, as is shown in the frequency distribution presented in Table 3 and the frequency polygon in Figure 2. There were 184 F_2 plants that began to produce spikelets as early as or earlier than the latest early-parent plant; 243 F_2 plants that began spikelet emergence between the dates of spikelet emergence on the latest early-parent plant and on the earliest late-parent plant; and 138 F_2 plants that were as late as or later than the range of the late-parent plants. Thus there was a heaping up of plants in the intermediate position, which was also observed to be the case in the F_2 population from the cross Niro Vialone × Caloro. In both of these crosses there were many more midseason and late than early plants in the F_2 segregations.

TABLE 3.—*Frequency distribution of the parents and F_2 progeny of the Eureka × Caloro rice cross by dates of first spikelet emergence in plants at Biggs, Calif., in 1925*

Date of emergence of first spikelet	Parents and progeny (number)			Date of emergence of first spikelet	Parents and progeny (number)		
	Eureka	Caloro	F_2 progeny		Eureka	Caloro	F_2 progeny
July 31			1	Aug. 23			5
Aug. 1			6	Aug. 24			1
Aug. 2			3	Aug. 25			23
Aug. 3	2		6	Aug. 26			23
Aug. 4	4		29	Aug. 27			40
Aug. 5	4		16	Aug. 28			35
Aug. 6	3		29	Aug. 29		7	23
Aug. 7	1		11	Aug. 30		24	35
Aug. 8	2		4	Aug. 31		6	24
Aug. 9			14	Sept. 1		2	18
Aug. 10	2		19	Sept. 2		1	9
Aug. 11			14	Sept. 3			12
Aug. 12			12	Sept. 4		1	8
Aug. 13	2		20	Sept. 5			1
Aug. 14			10	Sept. 6			6
Aug. 15			23	Sept. 7			0
Aug. 16			9	Sept. 8			0
Aug. 17			18	Sept. 9			1
Aug. 18			8	Sept. 10			0
Aug. 19			11	Sept. 11			1
Aug. 20			17				
Aug. 21			14	Total	20	41	565
Aug. 22			6				

F_3 PROGENY

In 1925, 47 F_3 families were grown from the cross Niro Vialone × Caloro, including early, midseason, and late progenies. These consisted of 18 families with purple leaves, 14 with green leaves, 9 with purple-striped leaves, and 6 with green leaves and red lemma tips. Each family was grown in four rod rows spaced 2 feet apart, and the plants were spaced about 6 inches apart in the rows. Notes were taken on the date of first and last spikelet emergence within each family. In nine families the date of first spikelet emergence

was taken for each plant, as well as the number of culms per plant, height of plant, length of panicles, and yield per plant. These 47 families also were used in a study of the inheritance of color and other contrasting characters.

TABLE 4.—*Frequency distribution of the parents and F_3 progeny of the Niro Vialone×Caloro rice cross by dates of first spikelet emergence in plants at Biggs, Calif., in 1925*

Date of emergence of first spikelet	Parents and progeny (number)											
	Niro Vialone	Caloro	221A22	A41	A59	A85	A14	A60	A50	A170	A12	Total progeny
July 30			2	1	2							5
July 31			2	1	1	1						5
Aug. 1			8	1	1	4	1					15
Aug. 2			28	8	4	5	2	1				48
Aug. 3			21	4	6	6	6		2			45
Aug. 4			9	2	2	6	5	1	1	3		29
Aug. 5			14	6	5	10	9	4	1	1		50
Aug. 6	4		24	4	6	5	18	2	3	3		65
Aug. 7	4		4	5	2	3	12	4	5	2		37
Aug. 8	3		5	10	3	10	5	2	2	4		41
Aug. 9	2		5	7	2	13	5	2	2	2		38
Aug. 10	4		1	7	5	9	6	2	4			34
Aug. 11			4	4	3	2	1	3		2		19
Aug. 12	2		2	6	1	7	9			2		27
Aug. 13			4	5	7	5	6	2	1	4		34
Aug. 14			2	1	8	4	3		1	5		24
Aug. 15	4				9	1	5	3		4		22
Aug. 16	2				1	2	3	2		9		17
Aug. 17	1		1	2	5	1	5	1		5		20
Aug. 18				1	3	3	2			2		11
Aug. 19				2	3	5	3		1	5		19
Aug. 20					2	2	4			1		9
Aug. 21					7	2	3	1		3		16
Aug. 22				1	2		4	1		1		9
Aug. 23				1		5		2		1		9
Aug. 24					5	4	3	1	1	6		20
Aug. 25				1	1	4		3		4		13
Aug. 26				2	1	4	3	3	2	8		23
Aug. 27				14	5	7		3	6	12	1	48
Aug. 28				11	4	4	1	9	11	5	4	49
Aug. 29		7		3	4	1		5	11	4	5	33
Aug. 30		24		3	10			3	10	6	8	40
Aug. 31		6		1	7			2	5	12	16	43
Sept. 1		2			1			3	2	7	14	27
Sept. 2		1			3			1		4	8	16
Sept. 3					1			1		2	20	24
Sept. 4		1									2	2
Sept. 5								2			7	9
Sept. 6									3		1	4
Sept. 7									5		1	6
Sept. 8									2		3	5
Sept. 9									2		1	3
Sept. 10									3			3
Sept. 11									5		1	6
Sept. 12									3		1	4
Sept. 13									1		1	2
Sept. 14									1		2	3
Sept. 15											1	1
Sept. 16									1		1	2
Sept. 17											1	1
Total	26	41	136	114	132	135	124	69	97	129	99	1,035

The frequency distribution for date of first spikelet emergence of the nine F_3 families and the parents is shown in Table 4 and the frequency polygons in Figure 3. It is shown in Table 4 that plants in eight of the nine F_3 families showed emergence of spikelets earlier than did the earliest of the early parent plants, and that in three families (including the ninth) there were plants later than the latest late-parent plant. There were plants in both families A50 and A60

that began spikelet emergence before any of the early parent plants, and also some that were later in spikelet emergence than the latest of the late-parent plants.

In all nine families there was transgressive inheritance with respect to date of first spikelet emergence. Family A22 bred almost true for earliness; that is, all plants in this family began to produce spikelets as early as did those of the early parent, and some plants were earlier. Family A12 was late in maturing, and some few plants were later than the late parent. Families A85, A14, and A59, if the plants were separated into two groups, using August 17, 18, and 23, respectively, as division dates, would show ratios of approximately 3 early to 1 late plant. In the three families there are 287 early to 104 late plants, or a deviation from a 3 : 1 ratio of 6.25 plants with a probable error of ±5.78. In families A60, and A170, if the plants are grouped as early and late, using August 19 and 22 as the respective division dates, there are in each case approximately 9 late to 7 early plants. In family A41, when August 15 is used as a dividing date, there are 72

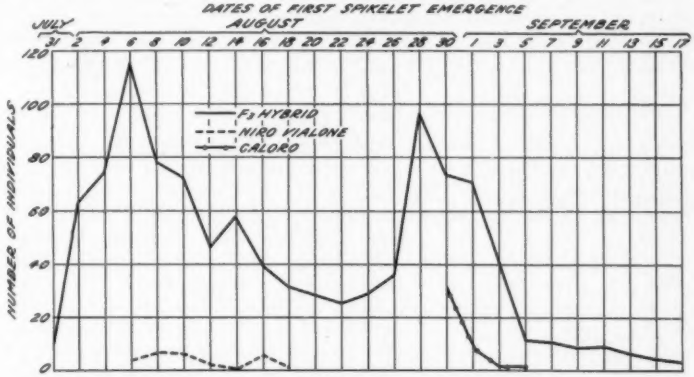

FIG. 3.—Frequency polygon showing dates of first spikelet emergence for the plants of the parent varieties and of nine F_3 families of the Niro Vialone×Caloro rice cross grown at Biggs, Calif., in 1925

early to 42 late plants, a not very close approximation of 9 early plants to 7 late.

The segregation of family A50 is distinctly into three groups in a ratio of 1 early to 2 midseason to 1 late plant. The deviation from the calculated numbers in a 1 : 2 : 1 ratio is very small for each group. This is the most distinct segregation obtained in the F_3 families.

F_4 PROGENY

Selections of mostly early, but a few midseason, plants were made from 47 F_3 families in the fall of 1925, and 355 of these selections from 44 F_3 families were grown in 1926. In Table 5 are given the date of first spikelet emergence for the F_2 plants grown in 1924 and the approximate date of first spikelet emergence for the F_3 and F_4 families grown in 1925 and 1926, respectively. In 1924 the notes are from single F_2 plants; in 1925 from a family consisting of four rod rows with plants spaced approximately 6 inches apart; and in 1926 from 1 to 10 families grown in rod rows and derived from single plants selected in 1925.

TABLE 5.—*Date of first spikelet emergence of F_2 plants in 1924 and of first and last spikelet emergence of F_3 and F_4 progeny in 1925 and 1926, respectively, from the rice cross Niro Vialone×Caloro, at Biggs, Calif.*

[T=Breeding true or almost true; S=segregating for dates of maturity; T-S=both true and segregating]

Color group and No. of family	Date of first spikelet emergence of F_2 plants in 1924	Date of first and last spikelet emergence				Notes
		F_3 families in 1925		F_4 families in 1926		
		First	Last	First	Last	
Purple leaves:						
22A4	Aug. 2	Aug. 1	Aug. 28	July 21	July 27	T.
22A22	July 31	do	Aug. 17	do		T.
22A43	Aug. 2	Aug. 2	Aug. 27	July 24	July 31	T.
22A61	July 28	July 31	Aug. 10	July 21	July 24	T.
22A26	Aug. 2	Aug. 2	Aug. 18	July 24	July 31	T.
22A69	Aug. 3	do	do	July 27	do	T.
22A110	July 31	July 31	Aug. 19	do	Aug. 2	T.
22A114	July 28	July 29	do	July 21	July 24	T.
22A63	Aug. 9	July 31	Aug. 29	do	Aug. 18	T-S.
22A80	Aug. 7	do	Sept. 1	do	Aug. 21	T-S.
22A83	Aug. 10	Aug. 2	do	July 24	Aug. 18	T-S.
22A85	Aug. 5	July 31	Aug. 29	July 21		S.
22A28	Aug. 8	do	Sept. 1	do	July 27	T-S.
22A35	do	Aug. 2	do	July 24	Aug. 23	T-S.
22A29	Aug. 12	Aug. 5	do	July 28	July 31	T-S.
22A23	Aug. 13	Aug. 3	Sept. 10			
22A60	Aug. 11	Aug. 2	Sept. 5			
22A154	Aug. 16	Aug. 15	Sept. 19	Aug. 4	Aug. 25	T-S.
Green leaves:						
22A14	Aug. 3	Aug. 1	Aug. 28	July 27		T.
22A165	Aug. 4	Aug. 2	Aug. 18	July 24	July 28	T.
22A15	Aug. 9	Aug. 14	Aug. 30	Aug. 8	Aug. 16	T.
22A51	do	Aug. 2	Aug. 20	July 24	Aug. 7	T.
22A190	Aug. 4	Aug. 1	Aug. 17	do	July 31	T.
22A54	Aug. 12	Aug. 7	Aug. 29	July 31	Aug. 8	T-S.
22A170	do	Aug. 4	Sept. 3	July 28		T.
22A173	Aug. 11	Aug. 6	Sept. 5	July 29	Aug. 12	T-S.
22A1	Aug. 9	Aug. 1	Sept. 14	July 27	July 31	S.
22A13	Aug. 10	Aug. 3	Sept. 1	July 28	Aug. 25	T-S.
22A37	Aug. 12	Aug. 6	Sept. 19	July 27	Aug. 5	T-S.
22A12	Aug. 15	Aug. 27	Sept. 17	do	July 31	T.
22A42	Aug. 14	Aug. 3	Sept. 1			
22A140	Aug. 19		Sept. 20	Aug. 25	Aug. 27	T.
Purple-striped leaves:						
22A88	July 26	July 28	Aug. 17	July 21	July 27	T.
22A38	Aug. 5	Aug. 2	Sept. 1	do	do	T-S.
22A163	Aug. 4	Aug. 4	Aug. 17	July 24	July 31	T.
22A174	do	Aug. 3	Aug. 19	do	do	T.
22A81	Aug. 6	Aug. 1	Sept. 1	July 17	Aug. 17	T-S.
22A30	Aug. 4	Aug. 4	Aug. 19	July 24	July 27	T.
22A59	Aug. 6	July 30	Sept. 3	July 21		T.
22A167	Aug. 10	Aug. 5	Sept. 18	July 24	Aug. 16	T-S.
22A179	Aug. 12	Aug. 2	Sept. 1	July 31	Aug. 25	T-S.
Green leaves, red lemma tips:						
22A41	Aug. 6	July 30	Aug. 31	July 21		T.
22A168	Aug. 2	Aug. 2	Aug. 13	July 27	July 31	T.
22A45	Aug. 9	do	Sept. 9	do	Aug. 12	T-S.
22A133	Aug. 11	do	Sept. 14	do	July 31	T-S.
22A50	Aug. 12	Aug. 3	Sept. 15	do		T.
22A172	Aug. 15	Aug. 28	Sept. 18	Aug. 16	Aug. 21	T-S.

The F_2 plants grown in 1924 were first irrigated on May 9 and the F_3 and F_4 families grown in 1925 and 1926 were first irrigated on May 16 and May 7, respectively. In Table 5 it is observed that nearly all F_2 plants have given rise to earlier forms in F_3 and F_4, which indicates that all F_2 plants in this case were heterozygous with respect to earliness.

In 1926 there was a total of 203 F_4 selections derived from single F_3 plants that bred true or almost true for early maturity, 41 that bred true or almost true for midseason maturity, and 111 that segregated for early and midseason plants.

A number of the early-maturing plant selections made in F_3 and grown in F_4 bred true or almost true for early maturity, and some of the midseason selections also bred true or nearly true in F_4. True-breeding strains from 7 to 10 days earlier than the early parent (Niro Vialone) were obtained in F_4. Some strains, however, continued to segregate in F_4 for early and midseason maturity.

The data herein presented on the inheritance of earliness in rice in the F_1, F_2, F_3, and F_4 generations indicate that, while earliness probably is inherited in the same manner as other quantitative characters, the F_2 population studied could not be placed with any degree of certainty in any of the simple or modified Mendelian ratios. Transgressive inheritance occurred and there was a heaping up of the plants in the intermediate position in F_2 populations. In the F_3 progeny one family bred almost true for early maturity and one almost true for late maturity. Three other families segregated in a ratio of approximately 3 early to 1 late plant, and one family produced plants in a distinct ratio of 1 early to 2 midseason to 1 late. Other families did not appear, however, to segregate in any definite ratio, for the segregating plants were distributed somewhat like the F_2 plants. The data presented indicate that two or more genetic factors are involved in the production of earliness in the rices studied.

LENGTH OF GROWING PERIOD

The average number of days from the date of first irrigation to maturity for the Niro Vialone and Caloro varieties during the six-year period from 1918 to 1923, inclusive, was 134 and 154 days, respectively. Niro Vialone therefore is normally about 20 days earlier than Caloro.

The mean number of days to maturity of the parent plants and the F_2 progeny, with the standard deviation and coefficient of variation of the F_2, are given in Table 6. It is observed that the parent varieties were mature in 129 and 143 days, respectively, from the date of the first irrigation. The fact that the parent varieties required less time in 1924 to reach maturity than the average number of days given for 1923 probably was due to the fact that they were started in a lighter soil which was warmer and more favorable for germination and early growth. It should also be remembered that the parent plants were transplanted in 1924, and this, too, may have induced earlier maturity. Climatic factors from year to year also have a marked influence upon the date of maturity of a given variety. In 1924 rice ripened rapidly, due apparently to the favorable temperatures and low humidity.

TABLE 6.—*Mean length of growing season in days, standard deviation, and coefficient of variation for days to maturity of plants of F_2 hybrids and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924*

Material	Number of plants	Mean number of days to maturity	Standard deviation	Coefficient of variation
F_2 hybrid	207	135.64±0.413	8.786±0.292	6.477±0.215
Niro Vialone	32	129		
Caloro	34	143		

The mean length of the growing period of the F_2 progeny was 135.6 days. This is almost exactly the average of the parent varieties, which was 136 days. The standard deviation of the F_2 progeny shows considerable variability with respect to length of growing period, but this is to be expected in a heterozygous population.

The data on the mean length of the growing period of the F_3 plants and parent varieties are shown in Table 7. The mean length of this period for the F_3 plants was intermediate between that of the parents, but somewhat nearer the late parent, Caloro. The standard deviation indicates that the F_3 plants were much more variable in respect to

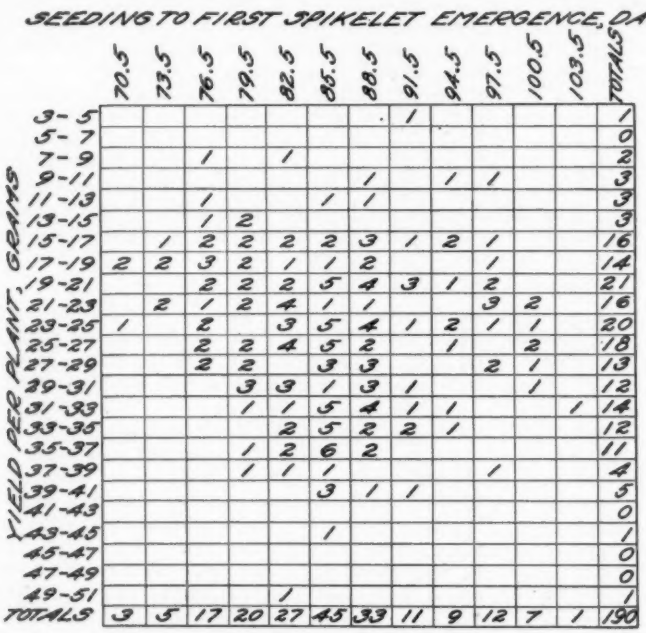

FIG. 4.—Correlation table for 190 F_2 rice plants of the cross Niro Vialone×Caloro grown at Biggs, Calif., in 1924. Total yield of plant in grams, subject. Number of days from first irrigation to first spikelet emergence, relative. $r_{xy}=0.0631\pm0.049$

date of maturity than were the parents and that the Caloro parent was much less variable than was the Niro Vialone parent.

TABLE 7.—*Mean length of growing season in days, standard deviation, and coefficient of variation for days to maturity of plants of F_3 hybrids and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925*

Material	Number of plants	Mean number of days to maturity	Standard deviation	Coefficient of variation
F_3 hybrid	470	139.13±0.212	6.823±0.150	4.904±0.108
Niro Vialone	26	128.69± .317	2.398± .224	1.863± .174
Caloro	32	146.00± .067	.559± .047	.383± .032

In connection with date of maturity it is of interest to know whether there is a correlation between date of first spikelet emergence, or flowering, and plant yield. In Figure 4 is given a correlation table for 190 F_2 rice plants from the cross Niro Vialone × Caloro. Total yield per plant in grams is subject. Number of days to first spikelet emergence in 1924 is relative. In Figure 4, $Rxy = 0.0931 \pm 0.049$, which shows that there apparently is no correlation between the yield of plant and number of days to first spikelet emergence. The fact

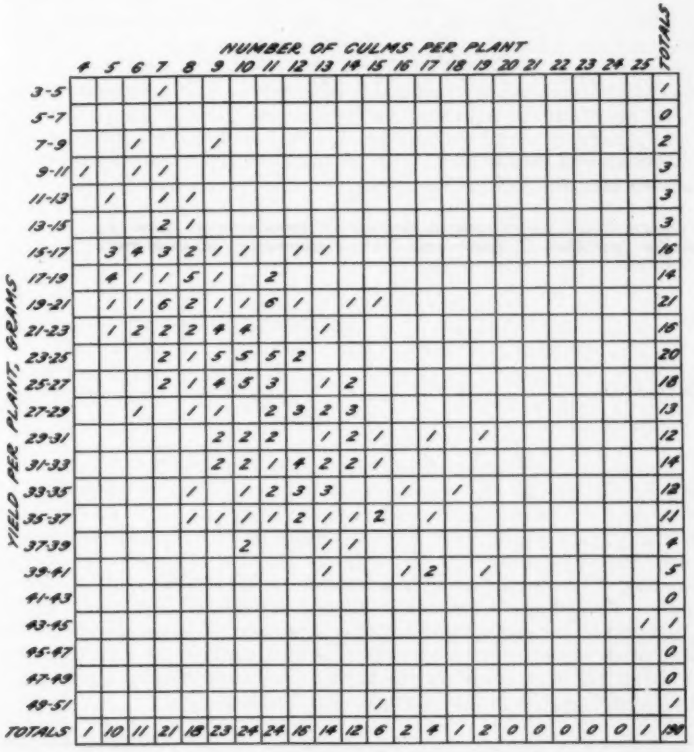

FIG. 5.—Correlation table for 190 F_2 rice plants of the cross Niro Vialone × Caloro grown at Biggs, Calif., in 1924. Total yield of plant in grams, subject. Number of culms per plant, relative. $Rxy = 0.670 \pm 0.027$

that these two characters are not correlated indicates that it may be possible to secure by recombination of characters an early-maturing, high-yielding rice.

There was a positive and significant correlation between the yield per plant and the number of culms per plant, $Rxy = 0.670 \pm 0.027$, as is shown in Figure 5. There was no correlation, however, between the yield per plant and the length of panicles, $Rxy = -0.080 \pm 0.049$. Likewise, the yield per plant and height of plant were not correlated, $Rxy = 0.0601 \pm 0.049$.

INHERITANCE OF YIELD

Yield undoubtedly is affected by the morphological and physiological characters of the plant as well as by the environmental complex. Engledow and Wadham (*3*) state:

> Cereal yield is controlled by a great number of factors which are themselves complex and imperfectly understood. In approaching the "yield problem" it is convenient to arrange these in broad categories which may thus be designated: (i) Soil, (ii) Climate, (iii) Agricultural practice, (iv) Disease and Damage, (v) Botanical variety or form.

The yields of F_2 plants and of the parents were obtained in the Niro Vialone × Caloro cross. The F_2 and parent plants, as stated before, were grown in rows spaced 3 feet apart, and the plants were spaced approximately 6 inches apart in the rows. The panicles were cut from the standing plants at maturity, and those of individual plants were placed in large manila envelopes. The envelopes were stored in the laboratory for about two months before the panicles were hand threshed and the grain weighed.

TABLE 8.—*Mean yield, standard deviation, and coefficient of variation for yield of plant of F_2 hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924*

Material	Number of plants	Mean yield (grams)	Standard deviation	Coefficient of variation
F_2 hybrid	190	25.16±0.377	7.708±0.267	30.636±1.155
Niro Vialone	30	19.87± .603	4.894± .426	24.630±2.271
Caloro	32	24.06± .652	5.465± .461	22.714±2.011

The mean yield, standard deviation, and coefficient of variation for the F_2 plants and the parent varieties are given in Table 8. It is seen that the mean plant yield of the F_2 progeny is greater than that of the parent varieties. The standard deviation and coefficient of variation show that the F_2 progeny were more variable than the parents. Yields of 470 F_3 plants from nine families were obtained in 1925. The plants were grown in rows spaced 2 feet apart, and the plants were spaced about 6 inches apart in the rows. Only plants that were spaced approximately 6 inches apart were harvested for a study of the agronomic characters. The data obtained on yield for the F_3 and parent plants are given in Table 9.

TABLE 9.—*Mean yield, standard deviation, and coefficient of variation for yield of plant of F_3 progeny and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925*

Material	Number of plants	Mean yield (grams)	Standard deviation	Coefficient of variation
F_3 hybrid	470	25.76±0.242	7.780±0.171	30.202±0.722
Niro Vialone	26	45.46±1.076	8.131± .760	17.886±1.726
Caloro	32	45.73± .892	7.484± .631	16.366±1.416

The F_3 plants included early, midseason, and late forms, and all nine F_3 families from which the 470 plants were harvested were segregating for earliness. Many of the late and some of the midseason

F_3 plants did not set seed and ripen well as a result of unfavorable blooming and ripening weather. Yields of the F_3 plants were quite variable, therefore, and lower than would be expected under more favorable climatic conditions.

Inspection of Table 9 shows that the mean yields of the parent varieties for the limited number of plants grown were not significantly different. The mean yield of the F_3 plants, however, was significantly lower than that of the parents. Using the coefficient of variation as a measure of variability, it is noted that the F_3 plants were much more variable than either parent, Caloro being slightly less variable than Niro Vialone.

INHERITANCE OF PLANT HEIGHT

Height of plant is an important character in rice, because the rice crop in the United States is harvested with grain binders. It is an essential requirement, therefore, that commercial varieties grow tall enough to bind well. In this study the height of the F_2 generation and of the parent varieties was measured at maturity. Height was measured from the base of the culms to the tip of the glume of the uppermost spikelet.

The mean height, standard deviation, and coefficient of variation for height of plant in F_2 progeny and for the parent varieties is shown in Table 10. The parent varieties are approximately of the same height and show no significant difference in the amount of variation. However, the Caloro parent was a little more variable than the Niro Vialone parent, as is shown by the coefficient of variation. The mean height of the F_2 plants is greater than that of the parents. The difference in mean height of the F_2 plants and the parents is probably significant in each case, for it is more than six times the probable error. The F_2 plants also were considerably more variable with respect to height than either parent.

TABLE 10.—*Mean height, standard deviation, and coefficient of variation for height of plant of F_2 hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924*

Material	Number of plants	Mean height (inches)	Standard deviation	Coefficient of variation
F_2 hybrid	207	36.69±0.142	3.019±0.100	8.228±0.273
Niro Vialone	32	35.44± .143	1.197± .101	3.378± .285
Caloro	34	35.50± .144	1.243± .102	3.501± .286

The data on the height of F_3 plants are shown in Table 11. The F_3 progeny are significantly shorter than either parent, but tall enough to bind well. The standard deviation and coefficient of variability show that the F_3 plants were much more variable in height than was either parent. This indicates that tall or short strains may be obtained if desired. The fact that the F_2 hybrid plants were taller than the parents and the F_3 plants shorter than the parents may indicate that heterosis influenced the height of the F_2 plants.

TABLE 11.—*Mean height, standard deviation, and coefficient of variation for height of plant of F_3 hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925*

Material	Number of plants	Mean height (inches)	Standard deviation	Coefficient of variation
F_3 hybrid	470	34. 57±0. 093	2. 993±0. 066	8. 658±0. 190
Niro Vialone	26	42. 77± . 128	. 971± . 091	2. 270± . 212
Caloro	32	38. 97± . 236	1. 976± . 167	5. 071± . 428

INHERITANCE OF NUMBER OF CULMS

Usually those varieties of rice which have a large number of culms per plant produce higher yields per plant and, under certain conditions, higher yields per acre than those varieties which do not tiller freely. Because of this relationship between yield and number of culms it is important to keep the stooling ability of plants in mind in a study of this nature. The number of culms in the F_2 and parent plants of the cross Niro Vialone × Caloro were recorded. The mean number of culms, standard deviation, and coefficient of variation for number of culms of the F_2 and the parent plants are given in Table 12.

TABLE 12.—*Mean number of culms, standard deviation, and coefficient of variation for number of culms per plant of F_2 hybrids and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924*

Material	Number of plants	Mean number of culms	Standard deviation	Coefficient of variation
F_2 hybrid	207	10. 48±0. 167	3. 555±0. 118	33. 922±1. 247
Niro Vialone	32	6. 19± . 306	2. 567± . 216	41. 470±4. 053
Caloro	34	14. 44± . 514	4. 440± . 363	30. 748±2. 742

In Table 12 it is interesting to note that the mean number of culms per plant for the Niro Vialone parent was 6.19, for the Caloro parent 14.44, and for the F_2 plants 10.48 culms per plant. The mean number of culms per plant of the F_2 progeny was almost exactly the average of the parents. Greater variation would be expected in the F_2 plants than in either parent, but the coefficient of variation shows that the Niro Vialone parent was more variable, while the Caloro parent was less variable than the F_2 progeny.

Data were obtained on the mean number of culms of 470 F_3 plants and the parent varieties, and these data are given in Table 13. The mean number of culms of the F_3 plants was significantly less than that of either parent. The coefficients of variation indicate that the F_3 plants were not significantly more variable with respect to number of culms than were the parents. The low mean yield obtained for the F_3 plants very probably is correlated with the small mean number of culms per plant.

TABLE 13.—*Mean number of culms, standard deviation, and coefficient of variation for number of culms per plant of F_2 hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925*

Material	Number of plants	Mean number of culms	Standard deviation	Coefficient of variation
F_2 hybrid	470	7.23±0.087	2.796±0.062	38.672±0.970
Niro Vialone	26	10.73± .539	4.072± .381	37.950±4.028
Caloro	32	13.03± .593	4.972± .419	38.158±3.656

INHERITANCE OF PANICLE LENGTH

The length of panicle in rice may or may not be closely associated with yield per plant. The length of the panicles of the F_2 plants and of the parents was recorded in this study. The panicles were measured in the laboratory, from the base of the panicle to the glume tips of the uppermost spikelet. The average length of all panicles on a plant was used as the panicle length for that particular plant. The mean panicle length for the parents and F_2 progeny, with the standard deviations and coefficients of variation, are shown in Table 14.

TABLE 14.—*Mean length of panicle, standard deviation, and coefficient of variation for length of panicle of plants of F_2 hybrid and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924*

Material	Number of plants	Mean length of panicle (inches)	Standard deviation	Coefficient of variation
F_2 hybrid	207	7.17±0.028	0.601±0.020	8.382±0.278
Niro Vialone	32	7.26± .030	.250± .021	3.444± .290
Caloro	34	6.51± .049	.427± .035	6.559± .536

Table 14 shows that the mean length of panicle of the early parent, Niro Vialone, was 18.45 cm. (7.26 in.), of the F_2 progeny 18.20 cm. (7.17 in.), and of the midseason parent, Caloro, 16.53 cm. (6.51 in.), respectively. In length of panicle the F_2 progeny are intermediate between the parents, but nearer the early parent, which had the longest panicles. In this connection it may be of interest to note that while the mean length of panicle for the early Niro Vialone parent was distinctly longer than that of the midseason Caloro parent, the latter produced a mean yield per plant of 24.06 gm., while the mean yield for the Niro Vialone plant was only 19.87 gm. The main reason for this is that the Caloro parent produced an average of about 14 culms per plant and Niro Vialone only about 6 culms per plant.

The coefficients of variation for length of panicle show that the F_2 progeny was more variable than either parent, the Niro Vialone parent being least variable with respect to panicle length. The greater variation of the Caloro parent no doubt is due in part to the tendency of midseason and late-maturing rice varieties to send out late tillers which often produce only short panicles. This formation of late culms is not nearly so common in early-maturing varieties of rice, which usually produce few culms bearing panicles of a comparatively uniform length.

Data on panicle length of the F_3 and parent plants are given in Table 15. It is observed that the panicles of the F_3 plants were significantly shorter than those of Niro Vialone and of about the same length as those of Caloro. The coefficients of variation indicate that the panicles of the F_3 plants were more variable in length than those of either parent.

TABLE 15.—*Mean length of panicle, standard deviation, and coefficient of variation for length of panicle of plants of F_3 hybrid and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925*

Material	Number of plants	Mean length of panicle (inches)	Standard deviation	Coefficient of variation
F_3 hybrid	470	7.29±0.018	0.593±0.013	8.134±0.179
Niro Vialone	26	7.52± .034	.259± .024	3.444± .322
Caloro	32	7.34± .041	.341± .029	4.646± .392

INHERITANCE OF SHAPE OF NECK

The elongated internode which bears the inflorescence of grasses is called the peduncle. That portion of the peduncle immediately below the panicle often is called the neck. In most rice varieties the neck is straight. Varieties with sinuous necks, however, are not uncommon.

In the Niro Vialone variety the neck is sinuous, while in Caloro the neck is straight. The F_1 hybrid from the cross Niro Vialone × Caloro had sinuous necks. In the F_2 progeny segregation occurred, producing 197 sinuous-necked plants to 10 plants with straight necks. This segregation suggests that duplicate factors are responsible for the sinuous-neck character, as is shown in Table 16; producing approximately a 15 : 1 ratio of sinuous-necked and straight-necked plants. The deviation from the calculated numbers in the 15 : 1 ratio is small and apparently not significant.

TABLE 16.—*Segregation of F_2 hybrids in the Niro Vialone×Caloro rice cross for sinuous-necked and straight-necked plants at Biggs, Calif., in 1924*

Shape of neck	Number of plants		Deviation from 15 : 1 ratio	Probable error [a]
	Observed	Calculated		
Sinuous	197	194.06	+2.94	±2.35
Straight	10	12.94	−2.94	2.35

[a] The probable errors for numbers of individuals given in this paper were obtained from tables of probable errors of Mendelian ratios prepared by the department of plant breeding, Cornell University, Ithaca, N. Y.

If the sinuous-neck character is due to dominant duplicate factors, as the F_2 data indicate, then in the F_3 generation there should be families that breed true for sinuous necks, others that breed true for straight necks, and still others that segregate in ratios of approximately either 3 or 15 sinuous-necked plants to 1 straight-necked.

Forty-seven F_3 families were grown in 1925, and the segregation of the heterozygous families is given in Table 17. Inspection of Table 17 shows that 14 families segregated in the ratio of 3 sinuous-necked

plants to 1 straight-necked, and 16 families segregated in the ratio of 15 sinuous-necked plants to 1 straight-necked. Four families, however, segregated in the ratio of about 9 sinuous-necked plants to 7 straight-necked. In 5 other families the ratio was about 1 sinuous-necked plant to 2 straight-necked ones. Eight F_3 families bred true for the sinuous-neck character.

TABLE 17.—*Segregation of 39 F_3 families of the Niro Vialone×Caloro rice cross for sinuous necks and straight necks at Biggs, Calif., in 1925*

[Symbols for neck shape: S=sinuous, St.=straight]

F_3 family No. and ratio	Shape of neck in F_2	Number of F_3 plants with necks—		Calculated on ratio named	Deviation	Probable error ±
		Sinuous	Straight			
Calculated on 3:1 ratio:						
221A1	S	91	25	29.00	−4.00	3.15
14	S	106	18	31.00	−13.00	3.25
29	S	88	19	26.75	−7.75	3.02
37	S	85	32	29.25	+2.75	3.16
38	S	73	36	27.25	+8.75	3.05
42	S	53	26	19.75	+6.25	2.60
43	S	73	17	22.50	−5.50	2.77
54	S	70	18	22.00	−4.00	2.74
59	S	88	45	33.25	+11.75	3.37
80	S	65	11	19.00	−8.00	2.55
140	St.	63	26	22.25	+3.75	2.76
165	S	66	13	19.75	−6.75	2.60
170	S	105	25	32.50	−7.50	3.33
172	St.	100	31	32.75	−1.75	3.34
Total		1,126	342	367.00	−25.00	11.17
Calculated on 15:1 ratio:						
221A4	St.	97	9	6.625	+2.375	1.68
22	S	133	3	8.500	−5.50	1.90
23	S	103	11	7.125	+3.875	1.74
26	S	106	14	7.500	+6.50	1.79
28	S	125	3	8.000	−5.00	1.85
35	S	83	11	5.875	+5.125	1.58
41	S	111	3	7.125	−4.125	1.74
61	S	135	2	8.5625	−6.5625	1.91
69	S	130	4	8.375	−4.375	1.89
110	S	110	4	7.125	−3.125	1.74
114	S	112	2	7.125	−5.125	1.74
133	S	93	1	5.875	−4.875	1.58
154	St.	79	5	5.250	−.250	1.50
167	S	96	5	6.3125	−1.3125	1.64
168	S	128	2	8.125	−6.125	1.86
190	S	110	3	7.0625	−4.0625	1.74
Total		1,751	82	114.5625	−32.5625	6.97
Calculated on 9:7 ratio:						
221A12	St.	52	47	43.3125	+3.6875	3.33
51	S	62	40	44.6250	−4.6250	3.38
85	S	77	58	59.0625	−1.0625	3.89
179	S	62	47	47.6875	−.6875	3.49
Total		253	192	194.6875	−2.6875	7.06
Calculated on 1:2 ratio:						
221A13	S	43	61	34.67	+8.33	.....
15	S	49	62	37.00	+12.00	.....
63	S	31	78	36.33	−5.33	.....
83	S	30	97	42.33	−12.33	.....
173	S	37	83	40.00	−3.00	.....
Total		190	381	190.33	−.33	7.60
221A30	S	96	.....	.....	.....	.....
45	S	111	.....	.....	.....	.....
50	S	102	.....	.....	.....	.....
60	S	69	.....	.....	.....	.....
81	S	115	.....	.....	.....	.....
88	S	70	.....	.....	.....	.....
163	S	106	.....	.....	.....	.....
174	S	126	.....	.....	.....	.....
Total		795	.....	.....	.....	.....

This neck character is rather difficult to classify correctly. Both senses, sight and touch, can be used to advantage in classifying the plants, and even then errors are very likely to occur. Climatic conditions may prevent the plants from shooting promptly, and often the leaf sheaths are so firmly clasped about the panicles that complete emergence of the panicle is prevented, and the necks in such cases often are bent and may be twisted, especially if climatic conditions are unfavorable during the shooting period, as they were in 1925 at Biggs.

It is probable that the four F_3 families segregating in the ratio of about 9 : 7 actually belong with those families that segregated in the ratio of 3 sinous-necked plants to 1 straight-necked. The five families that segregated in the ratio of about 1 sinous-necked plant to 2 straight-necked are difficult to dispose of and indicate that probably other disturbing factors are affecting the segregation for this character.

On the basis of F_3 breeding behavior, the F_2 ratio is changed from 197 sinuous-necked plants to 10 straight-necked to 202 sinuous-necked plants to 5 straight-necked. The deviation from calculated 15 : 1 ratio is 7.94 ± 2.35 plants. The deviation, therefore, is about 3.4 times its probable error and may be significant.

The fact, however, that 30 F_3 families produced expected ratios and 8 families bred true for sinuous necks indicates quite conclusively that at least two factors are involved in the production of the sinuous neck in the variety used in this study.

SUMMARY

This study was made on breeding material grown in an effort to obtain an early-maturing, high-yielding rice of good quality.

The Niro Vialone and Caloro varieties were used as parents in this cross because they appeared to be very good material for combining a study of the inheritance of contrasting characters with the possibility of obtaining in one variety early maturity, high yield, and good quality.

The cross was made and the material grown at Biggs, Calif.

F_1 hybrids from various rice crosses may be earlier than the early parent, later than the late parent, nearer the early or late parent, and the parents may be so different that the F_1 hybrids are sterile. The F_1 plant of the cross Niro Vialone × Caloro was nearer the early parent.

The segregation of F_2 progeny, from the crosses Niro × Caloro and Eureka × Caloro, for date of spikelet exsertion, shows that most of the plants were intermediate. In each case, however, transgressive inheritance occurred.

In the F_3 families of the cross Niro Vialone × Caloro there was one family that bred almost true for earliness, one family that was late maturing, three families that segregated in a ratio of about 3 early-maturing plants to 1 late, one family that gave a distinct ratio of 1 early to 2 midseason to 1 late, and other families that gave distributions somewhat like the F_2 hybrids.

Some of the early selections made from the F_3 progeny bred true in F_4. True breeding types were obtained that are from 7 to 10 days earlier than the early parent and are promising in yield and quality.

The F_2 hybrids were intermediate in date of maturity. Early and late-maturing F_3 selections were grown in 1925. Strains considerably earlier than the early parent were isolated.

There was apparently no correlation between the yield of F_2 plants and date of first spikelet exsertion. This indicates that high yield per plant and earliness may be combined in the same variety. Yields per plant and number of culms were definitely correlated, but length of panicles and height of plant were not correlated with yield per plant.

Yield is undoubtedly due to multiple factors. The F_2 hybrids exceeded the parents in yield and variability. The yields of the F_3 progeny were less than those of either parent, but were much more variable.

Tallness of plant appeared to be partially dominant in F_2, due probably to heterosis, the F_3 selections being significantly shorter than either parent.

In number of culms per plant the F_2 hybrids were almost exactly intermediate between those of the parents. The F_3 selections had fewer culms per plant than either parent, but were equally variable.

In length of panicle the F_2 hybrids were intermediate, but nearer the early parent, which has the longer panicles. The F_3 selections had shorter panicles than either parent. Variability was greater.

Sinuous necks were dominant to straight necks in F_1, and the F_2 and F_3 segregations suggest that at least duplicate genetic factors were probably involved.

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ILLUSTRATIONS OF THE APPLICATION OF A CRITERION OF THE DEVIATION OF AN OBSERVED FROM A RANDOM DISTRIBUTION TO THE PROBLEM OF SEEDLING STAND IN SEA-ISLAND, EGYPTIAN, AND UPLAND COTTON [1]

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INTRODUCTION

In practical agriculture an effort should be made to obtain in each hill planted an adequate number of seedlings to produce a full stand without waste of seed. Hills which contain no seedlings represent (in a field with proper spacing of hills) a waste of potentially productive land. Hills which contain a larger number of seedlings than is required to produce a full stand of suitable plants represent not merely a waste of seed in planting but result in too dense a stand in some places and necessitate the expense of thinning.

That the condition of the field as well as the quality of the seed influences the crop stand is well known to all agriculturists. Certain fields may, under the conditions of a particular season, produce stands so poor as to necessitate replanting, while other fields produce stands that are highly satisfactory. The physical causes of such differences may be clearly evident in a particular season, or they may be obscure.

In a single field the stand secured is often irregular. From inspection alone it may prove quite impossible to determine whether the observed irregularities are caused by diversities of the soil or other local influences or whether they are merely the result of the random distribution of seedlings when the stand as a whole is below the desired optimum.

It is impossible to hope for an entire absence of variation in seedling stand. The stand which most ideally meets practical needs would seem to be that in which the seedlings are distributed as uniformly as possible over the field. This will involve distribution in a random manner about a mean number such that practically no hills without seedlings and practically no hills with excessively large numbers of seedlings occur.

In work on disease resistance, in critical comparisons of the physiological characteristics of varieties, or in refined agronomic experiments of other kinds, it may sometimes become necessary to determine not merely the percentage germination which may be obtained in the field under given conditions but also the nature of the distribution of the stand over the field.

The specific problem here considered—that of the distribution of number of seedlings per hill—is highly complicated. Its final solution involves considerations of the germination rate, as such, of seeds exposed to field conditions, and the relationship of this rate

[1] Received for publication Jan. 3, 1928; issued June, 1928.

to the rate determined under laboratory conditions, of the physical factors of the soil and the physiological and morphological characteristics of the seedlings which may determine the emergence of the germinated seed from the soil, and of the relative susceptibility of different varieties to damping-off diseases. It is one phase of the general problem of field heterogeneity, which has been considered in a number of investigations for the Bureau of Plant Industry.

It is impossible to attack all these varied problems in one investigation, but it is reasonable to expect that the establishment of the existence of underlying causes of observable irregularities would necessitate as a first step the application of some criterion to test the significance of the deviation of the observed germination frequencies from a purely random distribution. It is the purpose of this paper to illustrate the use of a criterion applicable in certain cases, by concrete examples drawn from cultures of Egyptian, sea-island, and upland cottons as grown under irrigation at the United States Field Station of the United States Department of Agriculture and the United States Office of Indian Affairs at Sacaton, Ariz.[2] In this paper attention is limited to a comparison of the actual frequency distribution of the number of seedlings per hill with the theoretical distribution which should arise if frequencies were determined solely by chance.

METHOD OF DETERMINING THEORETICAL DISTRIBUTION OF NUMBER OF SEEDLINGS PER HILL

In determining the theoretical distribution of number of seedlings per hill the procedure is as follows:

Let s be the number of seeds planted per hill in N hills, g the number germinating per hill, and f the number failing to germinate per hill (or to survive to any stage desired). Then $s = g + f$.

Let $\bar{g}$ and $\bar{f}$ be the mean number of seeds germinating and failing to germinate, respectively, per hill for the tract as a whole. Then the chances, for the field as a whole, of germination, p, and that of failure, q, are defined as follows:

$$p = \frac{\bar{g}}{s} = \frac{\Sigma(g)}{Ns}$$

and

$$q = \frac{\bar{f}}{s} = \frac{\Sigma(f)}{Ns} = 1 - p$$

Now $p + q = 1$, and the distribution (assumed to be due solely to chance) of the relative frequencies when the number of seeds planted per hill is s (and is constant for the experimental tract under investigation) will be represented by the terms of the expression $(p+q)^s$, while the theoretical frequency for a given tract will be represented by the corresponding terms of $N(p+q)^s$, where N is the number of hills.

The significance of the divergence of the frequency distribution actually formed from the theoretical frequency distribution of number of seedlings per hill thus determined may be tested by means of Pearson's χ^2 criterion of goodness of fit,[3] employing Elderton's

[2] The writers thank C. J. King, superintendent of the station, most heartily for facilities provided and for his continued interest in the work.

[3] PEARSON, K. ON THE CRITERION THAT A GIVEN SYSTEM OF DEVIATIONS FROM THE PROBABLE IN THE CASE OF A CORRELATED SYSTEM OF VARIABLES IS SUCH THAT IT CAN BE REASONABLY SUPPOSED TO HAVE ARISEN FROM RANDOM SAMPLING. Phil. Mag. 50: 157–175. 1900.

tables [4] of the values of P to translate the values of χ^2 into terms of probability.

The simplest method of calculation may be illustrated by the entries for Pima cotton shown in Table 3.

The frequencies of germination of 0 to 6 seedlings per hill, respectively, in the 1,440 hills lead to $\Sigma(g) = 2{,}844$, $\bar{g} = 1.975000$,

$$p = \frac{\Sigma(g)}{6N} = \frac{\bar{g}}{6} = 0.329167,\ 1 - p = 0.670833$$

Table 1 shows the most convenient process of computing $N(p+q)^6$, which leads to the theoretical distribution in the final column.

TABLE 1.—*Calculation of theoretical distribution of number of seedlings per hill in Pima Egyptian cotton (experiment 3/22) as grown at Sacaton, Ariz., in 1922*

Powers of p	Powers of q	Products of powers of p and q	Coefficients	$(p+q)^6$	$N(p+q)^6$
	$q^6 = 0.091135$	$q^6 = 0.091135$	1	0.091135	131.2348
$p = 0.329167$	$q^5 = .135854$	$pq^5 = .044719$	6	.268312	386.3688
$p^2 = .108351$	$q^4 = .202515$	$p^2q^4 = .021943$	15	.329141	473.9625
$p^3 = .035666$	$q^3 = .301886$	$p^3q^3 = .010767$	20	.215339	310.0877
$p^4 = .011740$	$q^2 = .450017$	$p^4q^2 = .005284$	15	.079255	114.1276
$p^5 = .003864$	$q = .670833$	$p^5q^1 = .002592$	6	.015554	22.3980
$p^6 = .001272$		$p^6 = .001272$	1	.001272	1.8317
				1.000000	1,440.0000

A comparison of actual and theoretical frequencies in Table 2 leads to

$$S\left[\frac{(o-c)^2}{c}\right] = \chi^2 = 4372.7698$$

The number of decimal places retained throughout is larger than is required in most cases.

TABLE 2.—*Comparison of observed (o) and calculated (c) number of seedlings per hill in Pima Egyptian cotton and calculation of* χ^2

Seedlings per hill	Observed number of hills (o)	Calculated number of hills (c)	Observed minus calculated ($o-c$)	$\frac{(o-c)^2}{c}$
0	517	131.23	+385.77	1,134.0279
1	196	386.37	−190.37	93.7980
2	179	473.96	−294.96	183.5628
3	168	310.09	−142.09	65.1087
4	181	114.13	+66.87	39.1799
5	132	22.40	+109.60	536.2571
6	67	1.83	+65.17	2,320.8354
Total...	1,440	1,440.01	−000.01	4,372.7698

EXPERIMENTAL METHODS AND MATERIALS

The plantings were made by hand at the United States Field Station, Sacaton, Ariz., by careful workers experienced in experimental cotton culture. While the stand produced was low and the proportion of hills which failed to produce any seedlings was large, there was reason

[4] ELDERTON, W. P. TABLES FOR TESTING THE GOODNESS OF FIT OF THEORY TO OBSERVATION. Biometrika 1: 155-163. 1902.

to believe that the experimental technic could not have been improved.

Planting was standardized to six seeds per hill in all cases, in part because of the requirements of this phase of the investigations and in part because experience has shown that in the case of cotton grown under these conditions such a planting rate is necessary in order to obtain a reasonably full stand. This gives a possible range of 0 to 6 seedlings per hill. Seedling stand rather than germination rate serves as a basis for calculation, since the accurate determination of the number of seeds germinating under field conditions would be exceedingly difficult. Countings of number of seedlings were made when the plants had reached the stage of development at which thinning should be undertaken.

The data employed represent three types of cotton: Sea-island, Egyptian (Pima variety), and upland (Acala, Durango, Lone Star, and Meade varieties). The Pima Egyptian variety is also represented by a homozygous smooth-seeded form, while the Acala upland variety is also represented by a presumably mutant form known as okra-leaved Acala. These were grown in four experiments, the key numbers of which are here retained for convenience of reference.

EXPERIMENTAL DATA

Experiment 3/22 is a comparison of Pima Egyptian, Meade upland, and Acala upland cotton as grown in 1922. The organization of the experiment has been described in some detail in connection with a study of the relationship between the concentration of the soil solution and the physicochemical properties of the leaf-tissue fluids.[5] The properties of the soil and the physicochemical properties of the plant-tissue fluids have also been considered in connection with the problem of the nature of the regression of soil properties and crop characters in associated plots of the experimental field.[6] In brief, it represents plantings of 1,440 hills of each of these three varieties distributed over the north halves of the three adjacent plots E 3–1, E 3–2, and E 3–3, forming together a total area of 180 by 79.5 feet. The data are given in Table 3.

TABLE 3.—*Seedling stand in Pima Egyptian and Meade and Acala upland cotton as grown at the United States Field Station (plots E 3–1 to E 3–3, experiment 3/22), Sacaton, Ariz., in 1922*

Seedlings per hill	Number of hills		
	Pima Egyptian	Meade upland	Acala upland
0	517	645	412
1	196	279	236
2	179	181	186
3	168	174	242
4	181	98	200
5	132	44	125
6	67	19	39
Total	1,440	1,440	1,440

[5] HARRIS, J. A. THE RELATIONSHIP BETWEEN THE CONCENTRATION OF THE SOIL SOLUTION AND THE PHYSICOCHEMICAL PROPERTIES OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. Jour. Agr. Research 32 : 605–647, illus. 1926.

[6] HARRIS, J. A., CONNORS, I. L., ELDERS, A. T., and KIRK, L. E. ON THE REGRESSION OF SOIL PROPERTIES AND CROP CHARACTERS IN ASSOCIATED PLOTS OF AN EXPERIMENTAL FIELD. Minn. Univ. Studies Biol. Sci. 6: 351–371, illus. 1927.

Experiment 1/23 is a comparison of seedling stand in 1,440 hills each of Pima Egyptian and Lone Star upland cotton grown in 1923. The culture occupied an area of about 90 by 53 feet (omitting the area occupied by hybrid plants spaced between the two parent types and not here considered) on plots D 1–10 and D 1–11. The data are given in Table 4.

TABLE 4.—*Seedling stand in Pima Egyptian and Lone Star upland cotton as grown at the United States Field Station (plots D 1–10 and D 1–11, experiment 1/23), Sacaton, Ariz., in 1923*

Seedlings per hill	Number of hills	
	Pima Egyptian	Lone Star upland
0	850	879
1	171	165
2	104	112
3	107	113
4	97	89
5	73	64
6	38	18
Total	1,440	1,440

Experiment 1/25 represents a comparison of the seedling stand in Pima Egyptian cotton, smooth-seeded Pima Egyptian cotton, Acala upland cotton, and okra-leaved Acala upland cotton. The plantings were distributed over two different plots. Plot D 2–3 comprised an area of 360 by 26.5 feet and carried 720 hills of each of these four varieties. The frequency distributions of number of seedlings per hill are presented in Table 5. Plot D 1–10 was used in part for this experiment. The area used was 200 by 26.5 feet and carried 400 hills each of the four varieties. The frequency distributions appear in Table 6.

TABLE 5.—*Seedling stand in Pima Egyptian, smooth-seeded Pima, and Acala and okra-leaved Acala upland cotton as grown at the United States Field Station (plot D 2–3, experiment 1/25), Sacaton, Ariz., in 1925*

Seedlings per hill	Number of hills			
	Pima Egyptian	Smooth-seeded Pima	Acala upland	Okra-leaved Acala
0	211	154	341	347
1	49	33	72	95
2	62	55	75	82
3	108	65	92	72
4	137	129	69	64
5	109	153	52	43
6	44	131	19	17
Total	720	720	720	720

TABLE 6.—*Seedling stand in Pima Egyptian, smooth-seeded Pima, and Acala and okra-leaved Acala upland cotton as grown at the United States Field Station (plot D 1–10, experiment 1/25), Sacaton, Ariz., in 1925*

Seedlings per hill	Number of hills			
	Pima Egyptian	Smooth-seeded Pima	Acala upland	Okra-leaved Acala
0	51	56	121	116
1	34	22	85	59
2	37	34	58	62
3	56	41	54	72
4	80	82	36	46
5	82	87	35	31
6	60	78	11	14
Total	400	400	400	400

Since the purpose in investigating the distribution of number of seedlings per hill is to test the influence of extremely localized conditions—physical or chemical properties of the soil, inability of a given group of seedlings to break through the overlying mass of soil, or irregularities of distribution of an infecting organism—on seedling stand, these two series (plots D 2–3 and D 1–10) may be combined in order to give frequency distributions of 1,120 hills each. Since such distributions may be formed by combining the entries of Tables 5 and 6, they need not be published here. The table of constants (Table 11) gives the results of calculations based on the combined material as well as on the two individual series.

Experiment 2/25 is a comparison of Pima Egyptian, sea-island, and Acala, Durango, Lone Star, and Meade upland cottons, made in 1925 on plot D 2–4, comprising an area of 360 by 26.5 feet. In this experiment, plantings of Pima Egyptian and sea-island cotton were repeated for each variety of upland cotton. Thus there are available 240 hills each of both Pima Egyptian and sea-island cotton for each of the upland varieties (Acala, Durango, Lone Star, and Meade), which were represented by 240 hills each. The data are given in Tables 7 to 10, each of which presents the frequency distribution of seedlings per hill in Pima Egyptian and sea-island cotton immediately associated with each of the four upland varieties considered.

TABLE 7.—*Seedling stand in Pima Egyptian, sea-island, and Acala upland cotton as grown at the United States Field Station (plot D 2–4, experiment 2/25), Sacaton, Ariz., in 1925*

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Acala upland
0	40	46	83
1	15	13	42
2	25	20	37
3	41	34	37
4	54	40	28
5	46	55	10
6	19	32	3
Total	240	240	240

TABLE 8.—*Seedling stand in Pima Egyptian, sea-island, and Durango upland cotton as grown at the United States Field Station (plot D 2–4, experiment 2/25), Sacaton, Ariz., in 1925*

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Durango upland
0	47	68	63
1	16	14	17
2	30	15	15
3	36	23	28
4	48	34	42
5	49	50	50
6	14	36	25
Total	240	240	240

TABLE 9.—*Seedling stand in Pima Egyptian, sea-island, and Lone Star upland cotton as grown at the United States Field Station (plot D 2–4, experiment 2/25), Sacaton, Ariz., in 1925*

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Lone Star upland
0	61	52	103
1	12	14	37
2	20	19	46
3	33	23	38
4	56	50	11
5	40	51	5
6	18	31	----------
Total	240	240	240

TABLE 10.—*Seedling stand in Pima Egyptian, sea-island, and Meade upland cotton as grown at the United States Field Station (plot D 2–4, experiment 2/25), Sacaton, Ariz., in 1925*

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Meade upland
0	59	68	91
1	16	12	30
2	29	17	40
3	37	29	36
4	47	36	25
5	35	46	15
6	17	32	3
Total	240	240	240

Since the Pima Egyptian and sea-island plants were grown in comparison with four upland varieties, the Egyptian and sea-island records may be treated in four individual groups each based on 240 hills. These may be designated Pima with Meade, Pima with Lone Star, Pima with Acala, and Pima with Durango. A similar notation applies to the sea-island variety. Since both of these varieties (Pima Egyptian and sea island) were distributed over the whole

experimental area, the four subseries may be combined to give a larger frequency distribution for both Pima Egyptian and sea-island cotton. These are designated merely as Pima Egyptian and sea island in Table 11.

TABLE 11.—*Values of χ^2 test for closeness of agreement of observed and theoretical frequency distributions of number of seedlings per hill in sea-island, Egyptian, and upland cotton*

Experiment and variety	Plot	Table No.	N	χ^2
Experiment 3/22:				
Pima (Egyptian)	E 3-1, E 3-2, E 3-3	3	1,440	4,372.8
Meade (upland)	E 3-1, E 3-2, E 3-3	3	1,440	2,975.6
Acala (upland)	E 3-1, E 3-2, E 3-3	3	1,440	10,601.5
Experiment 1/23:				
Pima (Egyptian)	D 1-10, D 1-11	4	1,440	21,981.5
Lone Star (upland)	D 1-10, D 1-11	4	1,440	12,672.4
Experiment 1/25:				
Pima (Egyptian)	D 2- 3	5	720	2,080.3
Smooth-seeded Pima (Egyptian)	D 2- 3	5	720	4,803.9
Acala (upland)	D 2- 3	5	720	2,687.9
Okra-leaved Acala (upland)	D 2- 3	5	720	3,080.5
Pima (Egyptian)	D 1-10	6	400	1,148.5
Smooth-seeded Pima (Egyptian)	D 1-10	6	400	2,104.7
Acala (upland)	D 1-10	6	400	696.7
Okra-leaved Acala (upland)	D 1-10	6	400	626.8
Pima (Egyptian)	D 1-10, D 2-3	5, 6	1,120	3,523.8
Smooth-seeded Pima (Egyptian)	D 1-10, D 2-3	5, 6	1,120	7,053.4
Acala (upland)	D 1-10, D 2-3	5, 6	1,120	3,191.3
Okra-leaved Acala (upland)	D 1-10, D 2-3	5, 6	1,120	3,227.6
Experiment 2/25:				
Pima (Egyptian) with Meade (upland)	D 2-4	7-10	240	599.2
Pima (Egyptian) with Lone Star (upland)	D 2-4	7-10	240	789.3
Pima (Egyptian) with Acala (upland)	D 2-4	7-10	240	552.5
Pima (Egyptian) with Durango (upland)	D 2-4	7-10	240	542.4
Pima (Egyptian)	D 2-4	7-10	960	2,490.5
Sea island with Meade (upland)	D 2-4	7-10	240	1,275.1
Sea island with Lone Star (upland)	D 2-4	7-10	240	1,136.3
Sea island with Acala (upland)	D 2-4	7-10	240	1,053.9
Sea island with Durango (upland)	D 2-4	7-10	240	1,457.2
Sea island	D 2-4	7-10	960	4,933.2
Meade (upland)	D 2-4	7-10	240	319.5
Lone Star (upland)	D 2-4	7-10	240	132.1
Acala (upland)	D 2-4	7-10	240	240.7
Durango (upland)	D 2-4	7-10	240	1,033.2

DISCUSSION OF DATA

The frequency distributions of number of seedlings per hill are given in Tables 3 to 10. Space precludes the presentation of the frequencies calculated on the assumption of chance distribution and the numerical values of the differences between the observed and the theoretical frequencies. These are, however, presented graphically in Figures 1, 2, and 3, where the actual percentage frequencies are shown by the height of the rectangles, while the computed percentage frequencies are shown by the height of the solid dots on the scale of ordinates.

In all of these series, which represent a considerable range of varieties and of soil and other conditions which may influence seedling stand, the more central classes of the frequency distributions show a deficiency of observed as compared with theoretical frequencies, whereas the lower and upper extremes show a material excess of observed as compared with theoretical frequencies. It is clear that environmental conditions inherent in the soil or differences in the capacities of the small groups of seedlings planted in the various hills

for lifting the soil mass are such as to favor high percentage germination or seedling survival in some places and low percentage germination or survival in others.

A comparison of the observed and theoretical distributions by means of Pearson's χ^2 test of goodness of fit[7] in Table 11 shows values of χ^2 which are very large in comparison with those given in

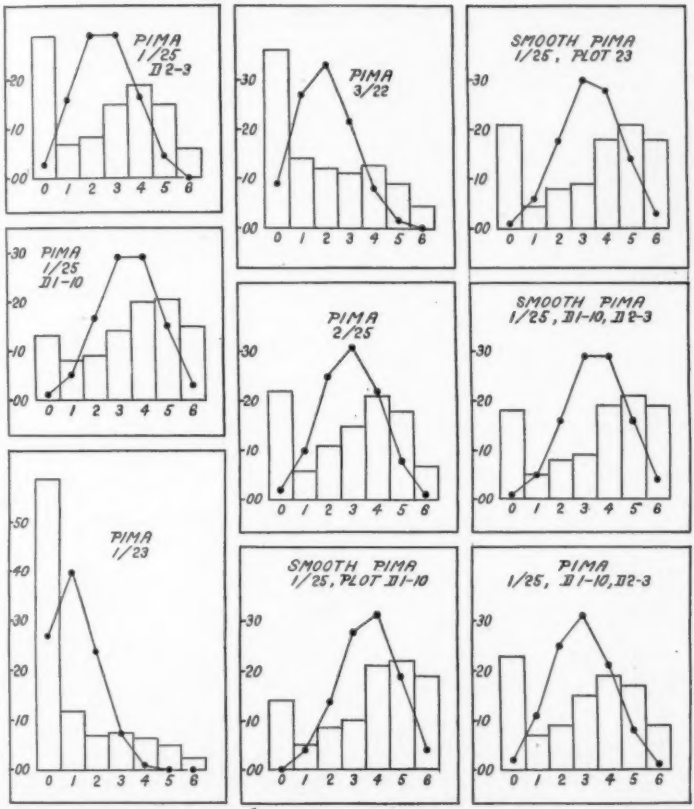
PIMA 1/25 II 2-3
PIMA 3/22
SMOOTH PIMA 1/25, PLOT 23
PIMA 1/25 II 1-10
PIMA 2/25
SMOOTH PIMA 1/25, II 1-10, II 2-3
PIMA 1/23
SMOOTH PIMA 1/25, PLOT II 1-10
PIMA 1/25, II 1-10, II 2-3

FIG. 1.—Comparison of observed (polygons) and computed (solid dots) frequencies of numbers of seedlings per hill, expressed as percentages, in Pima Egyptian cotton and smooth-seeded Pima Egyptian cotton, as grown at the United States Field Station, Sacaton, Ariz.

Elderton's table,[8] where P is the probability that a given system of deviations from the probable may be reasonably supposed to have arisen from random sampling.

The high values of χ^2 show that the probability that the deviation of the observed frequencies from a purely random distribution is, practically speaking, infinitesimally small. In other words, extremely localized conditions inherent in the environment or in the

[7] PEARSON, K. Op. cit.

[8] ELDERTON, W. P. Op. cit.

capacities of the small groups of six seedlings are, in all of these numerous experiments, of great importance in determining seedling stand.

SUMMARY AND CONCLUSIONS

This paper deals with the problem of the distribution of seedling stand over the agricultural field. Specifically, it indicates (1) a method for determining the theoretical number of seedlings per hill

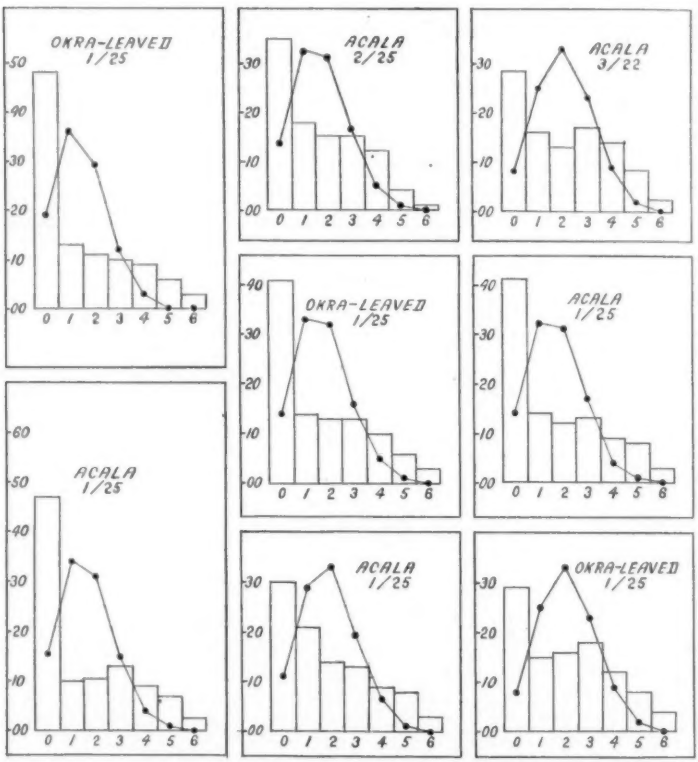

FIG. 2.—Comparison of observed (polygons) and computed (solid dots) frequencies of numbers of seedlings per hill, expressed as percentages, in Acala and okra-leaved Acala cotton, as grown at the United States Field Station, Sacaton, Ariz.

which should be found if the distribution were determined solely by the proportion of the seeds planted which produced plantlets and (2) a method of testing the significance of the difference between the observed and the theoretical distributions.

A suitable theoretical distribution for any field of N hills in which a constant number of seeds per hill, s, has been planted is given by the terms of $N(p+q)^s$, where $p=\frac{\Sigma(g)}{Ns}$ is the ratio of the number of seedlings produced and standing at the period of observation to the total number of seeds planted, and $q=1-p$.

In order to determine the closeness of agreement between any experimentally determined frequency distribution of number of seedlings per hill and the theoretical distribution, some criterion of goodness of fit is required. This paper suggests the use of the χ^2 criterion for that purpose.

The results of a comparison of observed and calculated frequencies of number of seedlings per hill in an extensive series of experimental plantings of cotton on the saline soils of the Gila River Valley at

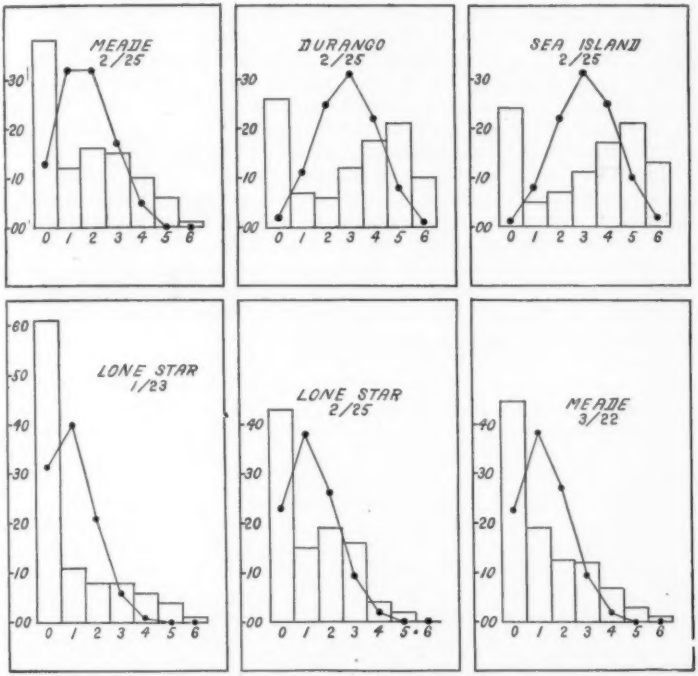

FIG. 3.—Comparison of observed (polygons) and computed (solid dots) frequencies of numbers of seedlings per hill, expressed as percentages, in sea-island cotton and in Durango, Lone Star, and Meade upland cottons, as grown at the United States Field Station, Sacaton, Ariz.

Sacaton, Ariz., show a material excess for the observed frequencies of hills without seedlings and of hills with larger number of seedlings. The application of the χ^2 test to these empirical and theoretical distributions shows that the chance of the difference between the two being due to random sampling is, practically speaking, infinitesimal.

It is clear, therefore, that the field conditions, or the samples of seeds planted in the individual hills, are highly heterogeneous with respect to factors influencing seedling stand. It is impossible, on the basis of the data presented here, to determine to what extent the observed variations in seedling stand and the observed deviations of the frequency distributions of seedling stand from the theoretical

distributions are due to soil conditions and to what extent they are due to the difficulty experienced by cotton seedlings in emerging. It is well known that, in order to secure a good stand of cotton, several seeds must be planted to a hill, since whenever the soil surface tends to form a crust the combined thrusting power of a number of seedlings is required to lift the crust and to insure the emergence of the plants. If most of the seeds planted in a particular hill chance to be nonviable, there may be too few seedlings to allow any of them to emerge. This factor is perhaps largely responsible for the excess over the expected proportion of hills containing no seedlings and of hills containing almost or quite the same number of seedlings as of seeds planted, as observed in the present investigation. But the properties of the soil which lead to the formation of a crust may differ from one part of the field to another. The peculiarities of distribution observed in this investigation may, therefore, in the last analysis, be an expression of soil heterogeneity.

That certain portions of the field may produce a better stand than others is of course quite in accord with conclusions based on general experience. As far as the writers are aware, however, quantitative tests of the significance of the deviation of an observed seedling stand from its random distribution have not heretofore been applied. Furthermore, the writers are unaware that such highly localized differences in stand as those indicated by the results here set forth have heretofore been demonstrated.

APPLICABILITY OF PEARSON'S EQUIVALENT PROBABILITY *r* METHOD TO THE PROBLEM OF SEEDLING MORTALITY IN SEA-ISLAND, EGYPTIAN, AND UPLAND COTTON[1]

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INTRODUCTION

In the biometric attack on the problems of agriculture, one of the objectives should be the determination of the relationship between various physical factors of the environment and crop yield or quality in terms of the correlation coefficient. This has not merely the advantage of expressing the relationships in which the agriculturist is interested in quantitative, mentally comprehensible, and universally comparable terms, but also that of permitting the determination of regression equations showing the relationship between the two or more variables under consideration in the concrete terms of the units of measurement employed.

Some progress has been made in this field of research by studies dealing with the problem of the prediction of crop yields from meteorological conditions prevailing during antecedent periods of time. In investigations conducted for the Office of Alkali and Drought Resistant Plants and the Office of Western Irrigation Agriculture, of the Bureau of Plant Industry, it has been shown that the relationship between the properties of the soil and certain plant characters may be measured directly in terms of correlation (*7*).[2]

In many cases, however, it is impossible to obtain an adequate quantitative measure of the whole complex of factors which influence profoundly crop yield or quality. In such cases it is necessary to obtain some measure of the influence of these variables in terms of (*a*) the correlation between the characteristics of associated cultures grown under varying conditions during the same year, by employing the method of intraclass or interclass correlation (*2*), or in terms of (*b*) the correlation between the yields or other characteristics of crops grown on the same land in different years, by employing the method of interannual correlation (*5*).

The method of intraclass correlation (*2*), in the form frequently designated as the field heterogeneity coefficient (*4*), has been employed in agricultural investigations which have led to papers on the measurement (*4*) and generality (*6*) of field heterogeneity. In these it has been shown that irregularities in the soil influence profoundly the characteristics of the crop grown. This has been found to be true not merely for yield but for such physicochemical properties

[1] Received for publication Jan. 3, 1928; issued June, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 623.

of the leaf-tissue fluids as osmotic concentration and electrical conductivity (*12*), chloride content (*13*), and sulphate content (*11*).

Confidence in the validity of this coefficient has recently been strengthened by the demonstration that the regression of both soil properties and plant characters of (small) associated plots is, practically speaking, linear (*9*). While the dangers of spurious correlation were early noted (*3*), formulas for determining the correlation from an asymmetrical surface while still utilizing the value of the intraclass and interclass moments have recently been indicated (*8*).

Application of the method of interannual (*5*) correlation to this group of problems has shown a relatively high degree of permanence in the yield-producing capacity of small experimental plots (*14*), although changes, which may be referred with reasonable probability to definite physical factors, may occur in the course of time (*15*).

While the above-mentioned methods are the best available for dealing with plot yields, a different procedure is necessary in investigating the influence of highly localized conditions (inherent either in a small sample of seeds or in the properties of the soil, such as those affecting a single hill) on seedling stand.

In a preceding paper (*10*, p. 604) the effect of such conditions on seed germination has been demonstrated by means of a method which, as far as the writers are aware, has not been applied heretofore in this field of work. This involved the calculation of a theoretical distribution of the number of seedlings per hill, on the assumption that there was no correlation between the viability of the seeds of the same hill or between the environmental conditions peculiar to the individual hills and the number of seedlings produced or surviving to any given stage of development. The difference between the theoretical and the empirical frequency distributions was then expressed in terms of Pearson's χ^2 criterion (*16*).

The application of this method to large series of germination records for sea-island, Egyptian, and upland cotton (*10*) led to values of χ^2 so large as to indicate that the probability of the differences between the empirical and the theoretical distributions having arisen from random sampling of the theoretical distributions is, practically speaking, infinitesimal. This shows clearly that the individual hills are highly differentiated with respect to their capacity for the production of seedling stand.

While this method of analysis is absolutely valid and leads to fully conclusive results, it has three disadvantages. First, the values of χ^2 secured in many experiments are so large that they can not be transmuted into values of P (the probability of the given distribution having arisen from random sampling) from Elderton's tables (*1*, *10*). Second, even if such probabilities were available in standard tables, they would be mentally incomprehensible to even highly trained individuals, the great majority of whom can not think in terms of such values as, for example, $137/10^{35}$. Third, such values, if laboriously computed, would be not merely incomprehensible but would express relationships in terms not directly comparable with those which have been secured in a number of other agricultural investigations.

The purpose of the present paper is to eliminate certain of the difficulties inherent in the preceding method of dealing with the problem, by applying to its analysis a procedure which brings the relationships under investigation within the scope of correlation methods (*17*).

METHODS

In an earlier paper (*10*) the authors determined the deviation of the actually observed frequency distribution of number of seedlings per hill from a theoretical frequency distribution. The present method also involves the deviation of observed from theoretical frequencies, but in the present case the deviations are those of the frequencies of two 2 by 2 fold tables of association.

The problem is, therefore, twofold. First, it is necessary to reduce a frequency distribution of number of seedlings per hill to a 2 by 2 fold table showing the distribution of the associations between seeds of the same hill. Second, it is necessary to determine the correlation between the fate of the seeds as potential producers of seedling stand from such a table.

In forming the fourfold table it must be noted that seeds of two categories are to be considered—those that survive to a given stage of development, *S*, and those that die before the given stage of development, *D*. Four combinations are therefore possible, *SS*, *SD*, *DS*, and *DD*. Thus the classes of the fourfold table representing the relationship of the fate of the seedlings of the same hill are as follows:

	Second seed		Total
	S	*D*	
First seed:			
S	*SS*	*SD*	*SS*+*SD*
D	*DS*	*DD*	*DS*+*DD*
Total	*SS*+*DS*	*SD*+*DD*	

Since there is no reason for regarding one seed of a hill as a first variate and another seed as a second variate of a pair, the table may be rendered symmetrical by making every possible permutation, two at a time, of the seedlings of an individual hill. Thus, if there be n seeds per hill, the number of possible permutations will be $n(n-1)$ in number; and if N be the total number of hills, the total frequency of a table representing the experimental plot as a whole will be $Nn(n-1)$.

Such tables may be readily formed from a frequency distribution of the number of seedlings surviving or dying in hills in which a given number of seeds were planted, as follows: Let n_s be the number surviving and n_d the number dying per hill; then $n_s+n_d=n$. The contribution of hills of any class to the fourfold table will then be $n_s(n_s-1)$ to *SS*, n_sn_d to *SD*, n_dn_s to *DS*, and $n_d(n_d-1)$ to *DD*. These values are readily tabulated for the several classes of hills, and their total contribution to a fourfold table computed by weighting

with the class frequencies. For example, the results for 400 hills of Pima Egyptian cotton of experiment 1/25 are given in the following tabulation:

Seedlings per hill	Number of hills	SS $n_s(n_s-1)$	SD n_sn_d	DS n_dn_s	DD $n_d(n_d-1)$
0	51	0	0	0	30
1	34	0	5	5	20
2	37	2	8	8	12
3	56	6	9	9	6
4	80	12	8	8	2
5	82	20	5	5	0
6	60	30	0	0	0

Summation of these values weighted with the number of hills leads to the symmetrical 2 by 2 fold table shown below.

	S	D	Total
S	4, 810	2, 020	6, 830
D	2, 020	3, 150	5, 170
Total	6, 830	5, 170	12, 000

For a given plot the probability of survival (p) of a seedling is given by the expression $\frac{SS+SD}{SS+SD+DS+DD}=\frac{SS+SD}{N}$ where N is the product of the number of hills and the total permutation frequency for one hill.

It is now possible to proceed to the construction of a theoretical 2 by 2 fold table in the usual manner, by assuming the random association of one variate with the other (in this case the variate with itself), i. e., by assuming that it is associated with the other variate in the same proportion that it occurs in the total population. Hence, if the entries of the theoretical table be designated by $S'S'$, $S'D'$, $D'S'$, and $D'D'$, the following relations are found:

$$S'S'=\frac{(SS+SD)\ (SS+DS)}{N}$$

$$D'S'=\frac{(DS+DD)\ (SS+DS)}{N}$$

$$S'D'=\frac{(SS+SD)\ (SD+DD)}{N}$$

$$D'D'=\frac{(DS+DD)\ (SD+DD)}{N}$$

It is now possible to calculate from first principles χ^2, which measures the deviation of the frequencies of a given table from those of the theoretical one. Thus:

$$\chi^2=\frac{(SS-S'S')^2}{S'S'}+\frac{(DS-D'S')^2}{D'S'}+\frac{(SD-S'D')^2}{S'D'}+\frac{(DD-D'D')^2}{D'D'}$$

Now, it may be noted that interest lies in the deviation of the sample in hand from one arising from random association and not in the theoretical table as such, and inasmuch as the prime values are given in terms of the original entries, it is possible by an algebraic conversion to calculate χ^2 directly in terms of them. Thus:

$$\chi^2 = \frac{N\,[(SS)\ (DD) - (SD)\ (DS)]^2}{(SS+SD)\ (DS+DD)\ (SS+DS)\ (SD+DD)}$$

The interpretation of χ^2 is given directly by Elderton's tables (*1, 18*) on a probability scale, i. e., these tables give the probability that the given distribution would have arisen from a random sampling of unassociated variates. As χ^2 grows large, however, P grows less readily comprehensible, and it was for this reason that Pearson (*17*) translated the interpretation of the deviation to the correlation scale. In principle, the method involves merely the determination or approximate determination of a correlation coefficient which would have the same probability of arising between two unassociated variables through random sampling.

Pearson (*17*) has shown that if r_{hk} be the correlation between the means of the two variates of a fourfold table on an assumption of Gaussian distribution of the variates, and ${}_o\sigma_{r_{hk}}$ its standard deviation on an assumption of absence of association, there is the following exact relationship:

$$\frac{r^2_{hk}}{{}_o\sigma_r{}^2_{hk}} = \frac{N\,[(SS)\ (DD) - (SD)\ (DS)]^2}{(SS+SD)\ (DS+DD)\ (SS+DS)\ (SD+DD)} = \chi^2$$

He has further shown that if ${}_o\sigma_r$ designates the standard deviation of the coefficient of correlation between the two varieties (designated by "r" as distinguished from the coefficient of correlation, r_{hk}, between their means) of a fourfold table, on the assumption that it is a random sample from uncorrelated material of Gaussian distribution, the following is approximately true:

$$\frac{{}_o\sigma_r}{r} = \frac{\sqrt{(SS+SD)\ (DS+DD)\ (SS+DS)\ (SD+DD)}}{\sqrt{N}\,[(SS)\ (DD) - (SD)\ (DS)]}$$

or approximately

$$\chi^2 = \frac{N\,[(SS)\ (DD) - (SD)\ (DS)]^2}{(SS+SD)\ (DS+DD)\ (SS+DS)\ (SD+DD)} = \frac{r^2}{{}_o\sigma^2{}_r}$$

Since interest centers in the association of the variates and not their means, it is necessary to deal with the latter relationship.

In determining the value of r from the relationship between χ^2 and $r^2/{}_o\sigma_r{}^2$, it is possible to take ${}_o\sigma_r = \frac{1}{\sqrt{N}} \times \chi_{\alpha_1} \times \chi_{\alpha_2}$, as given by a fourfold Gaussian table. χ_{α_1} and χ_{α_2} are the corrections of ${}_o\sigma_r$ for the failure of the table to have the material in equal-ranged cells. Here it is not necessary to deal with their technical significance any further than to point out that they are functions of $\frac{1}{2}(1+\alpha_1)$, $\frac{1}{2}(1-\alpha_1)$, $\frac{1}{2}(1+\alpha_2)$, and $\frac{1}{2}(1-\alpha_2)$ where $\frac{1}{2}(1+\alpha_1)$ and $\frac{1}{2}(1-\alpha_1)$

represent the two areas into which the frequencies of the variable designated by subscript 1 are divided when the total frequency is reduced to unity. In the present problem the variables are identical. Thus: $\alpha_1 = \alpha_2$, and the division is between S and D.

Thus $\frac{1}{2}(1+\alpha) = p$ if $p \geq 0.5$
$= 1-p = q$ if $p \leq 0.5$
and $\frac{1}{2}(1-\alpha) = 1-p = q$ if $p \geq 0.5$
$= p$ if $p \leq 0.5$

For values of $\frac{1}{2}(1+\alpha)$ given by a fourfold table, one may read off the corresponding χ_α's from Pearson's Table V (*17*) reprinted from Biometrika. For values of $\frac{1}{2}(1+\alpha)$ beyond two decimal places, it is necessary to interpolate between two tabular values of χ_α to determine the desired value.

With the value ${}_o\sigma_r$ thus determined and the value χ^2 calculated, it is possible to determine a value "r," which will satisfy the approximation

$$\chi^2 = \frac{r^2}{{}_o\sigma_r{}^2}$$

For this purpose Pearson has published two tables, one involving χ^2 and the other log. χ^2, of which the former, Table III (*17*), which gives values of χ^2 corresponding to values of r and ${}_o\sigma_r$, will be used here. In practice, this determination of r involves a double interpolation, one for values of χ^2 to correspond to values of ${}_o\sigma_r$ beyond two decimal places, and thereafter another interpolation between two tabulated values of r to obtain the one corresponding to a given χ^2. This procedure will be clear from a numerical example to follow.

The correlation coefficient thus obtained is an approximation of the tetrachoric r of the fourfold table, and subject to the same interpretation, with allowances. Allowance must be made not only for the approximation involved in the theory but for approximations involved in interpolation. There can be no doubt, however, that in spite of the approximations, this r presents to the mind a much more accurate impression of the degree of association than does the function P, representing the probability of there being no association. For purposes of distinction, and in view of its derivation and purpose, this correlation coefficient may be termed the "equivalent probability r."

As an example consider the "equivalent probability r" for the 400 hills of the plot of Pima Egyptian cotton in experiment 1/25, for which the tabulation on page 618 gives the fourfold distribution.

To calculate directly:

$$\chi^2 = \frac{N[(SS)(DD)-(SD)(DS)]^2}{(SS+SD)(DS+DD)(SS+DS)(SD+DD)}$$

$$= \frac{12000\,(11071100)^2}{(6830)(5170)(6830)(5170)} = 1179.6127$$

$$\tfrac{1}{2}(1+\alpha_1) = \tfrac{1}{2}(1+\alpha_2) = \tfrac{1}{2}(1+\alpha) = p = \frac{SS+SD}{N} = \frac{6830}{12000} = 0.569$$

Interpolating for χ_α one has:

$\frac{1}{2}(1+\alpha)$	χ_α
0.56	1.2585
0.569	1.2602
0.57	1.2604

$$\chi_\alpha = 1.2585 + 0.9\,(0.0019) = 1.2602$$

$$_o\sigma_r = \frac{1}{\sqrt{N}}\,\chi_{\alpha_1}\,\chi_{\alpha_2} = \frac{1}{\sqrt{N}}\,\chi_\alpha{}^2 = \frac{1}{\sqrt{12000}} \times (1.2602)^2$$

$$= 0.009129 \times 1.588104 = 0.0145$$

Interpolating values of χ^2 for $_o\sigma_r = 0.0145$ for values of $r = 0.4$ and $r = 0.5$, the values as indicated in the table opposite that entry are obtained.

$_o\sigma_r$	$\chi^2(r=0.4)$	$\chi^2(r=0.5)$
0.01	1758.21	2892.33
0.0145	1168.53	1920.19
0.02	447.81	731.95

Interpolation for the r corresponding to $\chi^2 = 1179.61$, gives:

χ^2	r_p
1168.53	0.4
1179.61	
1920.16	0.5

$$r_p = 0.4 + \frac{(0.1)\ 11.08}{751.63} = 0.4015$$

MATERIAL

The materials on which this study is based have been described in detail in another paper (*10*). The data comprise records of cotton stands from five different experiments with Pima Egyptian, sea-island, and upland cotton.

RESULTS

The results of the present rather onerous investigation may be condensed into a single accompanying table. (Table 1.) In this the original experiment numbers are retained. Reference to the frequency distributions of number of seeds per hill, as published in an earlier paper (*10*), is made by number in the second column. The number of hills appears in the third column. The permutations of seeds which survived and of those which died are indicated by the weighted frequencies in the fourth to seventh columns. The values of χ^2 appear in the eighth column, and the values of the equivalent probability r, as deduced from these values of χ^2, are given in the final column of the table.

For present purposes it has seemed unnecessary to consider the probable errors of these correlations. Without laying too much emphasis on the significance of the individual values of r_p, it is clear that these constants have material values. They range from $r = 0.31$ to $r = 0.75$. Thus there is at least a medium correlation between the fate of seedlings of the same hill.

TABLE 1.—*Permutation frequencies of number of seeds which survived (S), and number of seeds which died (D), in hills of sea-island, Egyptian, and upland cotton, together with values of χ^2 and equivalent probability correlation coefficients measuring the relationship between the fate of seedlings of the same hill*

Experiment and variety	Table No. (*10*)	Number of hills (*N*)	Survived and survived (*SS*)	Survived and died (*SD*)	Died and survived (*DS*)	Died and died (*DD*)	χ^2	*r*
Experiment 3/22:								
Pima Egyptian	3	1,440	8,188	6,032	6,032	22,948	5,839.7	0.6599
Meade upland	3	1,440	4,032	5,413	5,413	28,342	3,068.9	.5111
Acala upland	3	1,440	7,894	7,071	7,071	21,164	3,316.2	.5267
Experiment 1/23:								
Pima Egyptian	4	1,440	4,614	3,791	3,791	31,004	8,363.7	.7463
Lone Star upland	4	1,440	3,790	3,770	3,770	31,870	6,758.8	.7062
Experiment 1/25:								
Pima Egyptian	5	720	5,916	3,354	3,354	8,976	2,896.1	.5118
Smooth-seeded Pima Egyptian	5	720	9,038	2,987	2,987	6,588	4,175.0	.5976
Acala upland	5	720	3,140	2,600	2,600	13,260	3,170.2	.5590
Okra-leaved Acala upland	5	720	2,734	2,506	2,506	13,854	2,934.3	.5516
Pima Egyptian	6	400	4,810	2,020	2,020	3,150	1,179.6	.4015
Smooth-seeded Pima Egyptian	6	400	5,378	1,842	1,842	2,938	1,551.0	.4556
Acala upland	6	400	1,902	1,838	1,838	6,422	981.8	.3908
Okra-leaved Acala upland	6	400	2,148	1,962	1,962	5,928	921.5	.3683
Pima Egyptian	5,6	1,120	10,726	5,374	5,374	12,126	4,333.4	.5908
Smooth-seeded Pima Egyptian	5,6	1,120	14,416	4,829	4,829	9,526	5,722.2	.6547
Acala upland	5,6	1,120	5,042	4,438	4,438	19,682	4,065.8	.5740
Okra-leaved Acala upland	5,6	1,120	4,882	4,468	4,468	19,782	3,836.1	.5595
Experiment 2/25:								
Pima Egyptian with Meade upland	7–10	240	2,054	1,196	1,196	2,754	780.4	.4370
Pima Egyptian with Lone Star upland	7–10	240	2,250	1,165	1,165	2,620	887.4	.4590
Pima Egyptian with Acala upland	7–10	240	2,434	1,306	1,306	2,154	538.0	.3635
Pima Egyptian with Durango upland	7–10	240	2,252	1,273	1,273	2,402	615.9	.3902
All Pima Egyptian	7–10	960	8,990	4,940	4,940	9,930	2,824.3	.4940
Sea island with Meade upland	7–10	240	2,520	975	975	2,730	1,509.4	.5788
Sea island with Lone Star upland	7–10	240	2,726	1,084	1,084	2,306	1,127.5	.5124
Sea island with Acala upland	7–10	240	2,784	1,126	1,126	2,164	984.5	.4876
Sea island with Durango upland	7–10	240	2,656	919	919	2,706	1,724.6	.6113
All sea island	7–10	960	10,686	4,104	4,104	9,906	5,314.8	.6368
Meade upland	7–10	240	986	1,069	1,069	4,076	532.8	.4385
Lone Star upland	7–10	240	552	1,008	1,008	4,632	220.8	.3100
Acala upland	7–10	240	922	1,113	1,113	4,052	406.4	.3898
Durango upland	7–10	240	2,452	1,043	1,043	2,662	1,270.4	.5372

SUMMARY AND CONCLUSIONS

Earlier studies have dealt with the problem of the significance of the deviation of number of seedlings produced per hill in sea-island, Egyptian, and upland cotton, from the theoretical distributions which would result if the distribution of the stand were a purely random one.

The present paper indicates methods by which the deviations of the stand from a purely random distribution may be translated into terms of correlation between the fate of the seedlings of the same hill.

The stands obtained in a wide series of experiments show that there is a medium correlation between the fate of the seeds of the same hill. Thus, either extremely localized conditions or random differences in the capacities of the seeds of the various hills for establishing themselves as seedlings play a large rôle in determining variation in seedling stand.

The advantages of this method of attack are twofold. First, it expresses relationships in terms of correlations which are more readily comprehensible than those of large values of χ^2 and almost infinitesimally small values of P. Second, it permits the extension to this field of work of a combination of methods which have heretofore been employed in a wide range of field heterogeneity investigations.

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NUTRITIONAL STUDIES ON THE SEED-CORN MAGGOT, HYLEMYIA CILICRURA RONDANI [1]

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INTRODUCTION

The seed-corn maggot, *Hylemyia cilicrura* Rondani, is a pest of various crops, such as corn, beans, and potatoes, during the period of seed germination. The following investigation on the nutrition of the larva of this species sheds some particularly useful light on the question of the exact rôle of bacteria in the nutrition of the fly larva and clears up a question of much economic importance in regard to the biology of the species. From the economic point of view it was desired to learn whether or not the action of bacteria must precede the attack of the larva upon potato seed pieces, sprouting corn, and other plants susceptible to attack by the insect. From the theoretical standpoint it is important to know more about the food substances which are essential to larval development and which are made available by microorganisms.

HISTORICAL

Loeb and Northrop [3] have clearly shown the importance of yeasts in the nutrition of Drosophila larvae. They thought that yeasts were the indispensable food of these flies, and they proved that the value of the yeasts lay in their synthetic powers, for the larvae were able to live upon either living yeasts or yeasts which had been subjected to heat of 120° C. for one hour. Their attempts to extract the necessary substance from the yeast met with failure, and they were at a loss to explain the exact nature of this substance. Northrop [4] investigated the matter further and found that the specific substance essential to growth was also present in the kidneys, liver, and pancreas of the dog, in the liver of the mouse, and in the bodies of the flies themselves.

Leach [5] made similar studies on *Hylemyia cilicrura* (under the name *Phorbia fusciceps* Zett.). He found that bacteria were important in the normal development of these larvae and, in fact, went so far as to say that bacteria are essential for the development of the maggots. He found further that bacteria of the substratum, including some pathogenic species, when taken up by the larva of *Hylemyia cilicrura*, may live through the pupal stage and be disseminated by the adult fly. Since this condition makes possible the continuous associa-

[1] Received for publication Feb. 3, 1928; issued June, 1928.

[2] This investigation was conducted by the writer during the summer of 1927, while he was stationed at Chadbourn, N. C., as a temporary employee of the Bureau of Entomology. The writer wishes to express his thanks to W. H. White and to W. J. Reid, jr., for valuable aid in this work.

[3] LOEB, J., and NORTHROP, J. H. NUTRITION AND EVOLUTION. SECOND NOTE. Jour. Biol. Chem. 27: 309–312. 1916.

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tion of the maggot with the bacterial flora of the substratum, it appeared interesting and important to investigate further the food requirements of the larva with special reference to the rôle played by the bacteria present.

PRESENT INVESTIGATIONS

FOOD REQUIREMENTS OF THE LARVAE

Leach's conclusions were based upon experiments with sterile larvae placed upon sterile potato plugs and upon beef-extract agar. He observed that sterile maggots were unable to grow on sterile potato plugs, but that they grew and pupated on potato plugs which had been inoculated with bacteria found naturally in their environment. Repetition of Leach's experiments by the author yielded like results. No larvae grew to maturity on either of the two kinds of media when sterility was obtained. Normal growth and pupation occurred in a certain percentage of cases in which the potato plug was contaminated. The larvae grew on nonsterile beef-extract agar, but always died before pupation.

It was desired to know whether the inability of the larva to live upon sterile potato plugs was due to the destruction of enzymes by the high temperatures used in sterilization. To determine this a bacteria-free filtrate, obtained by passing the fluid from macerated tubers through a Berkefeld filter, was added to the sterile potato plugs. Repeated attempts to grow larvae on this medium were unsuccessful.

BACTERIA AS FOOD FOR THE LARVAE

Upon the supposition that the larvae might live upon bacteria which had been killed by heat, a heavy suspension of killed bacteria was added to sterile potato plugs. The organisms used were obtained by inoculating agar slants from cultures in which larvae grew normally, and, after an incubation period of 24 hours, making a suspension of the resulting growth in isotonic salt solution. Attempts to grow larvae on this medium were unsuccessful. There still remained the possibility that the heating of the bacteria destroyed some essential substance. Suspensions of living bacteria were filtered, using a Berkefeld filter, and the filtrate was added to a suspension of killed bacteria. Attempts to grow larvae on sterile potato plugs to which this mixture was added were also unsuccessful.

RELATION OF BACTERIA TO THE NUTRITION OF THE LARVA

After these experiments, it began to appear that the part played by the bacteria in the life of the larvae must be a secondary one, and that it probably consisted of the effects of the bacteria upon the substratum rather than of any peculiar qualities of the bacteria themselves. Hence, in the next experiments, potato plugs were inoculated with bacteria and incubated for several days until they were covered with an abundant, slimy growth. They were then sterilized by heat. When newly hatched, sterile larvae were placed upon this medium they grew rapidly, pupated normally, and produced adults which were normal in appearance. These larvae, pupae, and adults were all tested for bacterial sterility and were found to be sterile. These experiments proved conclusively that the

fly could live from egg to adult without the presence of living bacteria; and they indicated that the rôle of the bacteria was merely that of an agent for converting the potato tuber into available food for the larvae. These experiments were then modified by using fermented and partially decomposed beans and peas, which were later sterilized by heat. It was found that this latter medium was more satisfactory for routine use than that made from potato. A stock of it was thereafter kept on hand and used for rearing newly hatched larvae as soon as they had been tested for sterility. In this way it was possible to have on hand for other experiments sterile larvae of various known ages and of the corresponding sizes.[6]

ATTEMPTS TO GROW LARVAE IN THE ABSENCE OF BACTERIA

Having found that the larvae did not require living bacteria for their development, the writer believed that a method might be found for growing them in complete absence of any microorganisms, either dead or living. Upon the supposition that the action of the bacteria in making the substance of potatoes and beans available for use by the larvae consisted of some kind of digestion, various attempts were made to produce these changes without the presence of bacteria. Artificial digestion by saliva and by artificial pancreatic juice were first undertaken. Fresh saliva was filtered through a Berkefeld filter to obtain a filtrate free from bacteria, and this was added to sterile potato. The artificial pancreatic juice was prepared by using a powdered extract of pancreas. The powdered product was mixed with water and filtered through a Berkefeld filter. The bacteria-free filtrate was added to sterile potato. Attempts to grow larvae on such media were not successful. The addition of sterile dextrose water to the sterile potato plugs also failed to provide a suitable medium for growth.

In the next attempts to grow the larvae advantage was taken of the natural processes of digestion which occur when seeds germinate. As these digestive changes may go on in the absence of bacterial action, efforts were made to grow various seedlings to be used as a medium for larval growth under conditions of bacterial sterility. No very satisfactory method for obtaining a high percentage of bacterially sterile seedlings from a given number of seeds was found. Simple treatment of the seeds with bichloride of mercury solution (1 : 1,000) for 15 minutes, with subsequent rinsing with sterile water, gave a small percentage of sterile seedlings. After treatment the seeds were placed on sterile agar and allowed to germinate, and any showing growth of bacteria or molds within a week were discarded. When a seedling was found to be bacterially sterile, a sterile larva was placed upon it and observed from day to day. It was found that these larvae attacked the roots of sterile seedlings of corn, beans, peas, and radishes, and grew rapidly while living upon this medium. They pupated in the normal length of time, and the pupae produced adults which were normal in appearance. Sterility tests were made upon the larvae, pupae, and adults, and all of these stages failed to

[6] It should be said here that Bogdanov's [7] method of obtaining sterile larvae by treating the eggs with bichloride of mercury solution (1 : 1,000) for 15 minutes was found to be a very satisfactory routine method and was followed throughout these experiments.

[7] BOGDANOV, E. A. ÜBER DIE ABHÄNGIGKEIT DES WACHSTUMS DER FLIEGENLARVEN VON BAKTERIEN UND FERMENTEN UND ÜBER DIE VARIABILITÄT UND VARERBUNG BEI DEN FLEISCHFLIEGEN. Arch. Anat. u. Physiol., Physiol. Abt., 1908 (sup): 173–200. 1908.

produce bacterial growth when smeared on agar slants or crushed in beef-dextrose broth. By transferring the larvae from the sterile, decomposed bean medium it was possible to place a sterile larva at any stage of its life upon the seedling. When observed under the microscope the larva was seen to use its mouth hooks in tearing the plant tissue into shreds. No part of the bean seedling seemed to be exempt from attacks from the larva, but the cotyledons and leaves were usually not attacked until the larva had almost reached maturity. (Fig. 1, C and D.)

The procedure followed in testing for sterility was to allow the larva to pupate in the tube containing the seedling, then to transfer the puparium to another agar-slant tube into which had been placed a folded piece of sterile filter paper. The original tube was then incubated for 24 hours and examined. When the adult emerged, it was allowed to crawl over the surface of the agar and to deposit feces thereon. It was then removed and crushed in a tube of sterile dextrose beef broth. The tube containing the empty puparium and the broth tube were then incubated for 24 hours and examined for sterility. Control tests were run upon the corresponding stages of flies which were grown under nonsterile conditions. Such control tests always showed abundant bacterial growth while the other cultures remained sterile. (Fig. 1, A and B.)

It should be noted that the possibility that living symbionts may have been present in the egg and were passed to and continued to live in the later stages has not been disproved by these experiments, since these entities do not grow on ordinary bacteriological media.

ESSENTIAL FOOD SUBSTANCES

The results of these studies indicate that substances essential to life for *Hylemyia cilicrura* larvae may be obtained from potato plugs and from beans, peas, and other seeds following the growth of bacteria upon these media; and that suitable substances are also present in growing bean and other seedlings which have been free from bacterial action.

SUMMARY AND CONCLUSIONS

Leach's observations that sterile larvae do not grow to maturity on sterile beef-extract agar or potato plugs, whereas larvae grow normally on contaminated potato plugs, are confirmed by these studies.

Experiments using bacteria-free filtrate from unheated potatoes indicate that the failure of larvae to grow on heated potato is not due to the destruction of thermolabile substances such as enzymes.

Attempts to grow larvae on sterile potato to which had been added a suspension of heated bacteria were unsuccessful.

Potato plugs, beans, and peas which had partially decomposed and then were sterilized by heat proved to be good media for growth. Flies were reared from egg to adult on such media and found to be bacterially sterile.

Attempts to grow larvae on potato which had been submitted to artificial digestion by saliva and pancreatic juice were unsuccessful.

Growing bean and pea seedlings free from bacteria were found to provide a suitable medium for growth, flies being reared from egg to adult on such seedlings without the presence of bacteria, either dead or living.

FIG. 1.—A, Broth culture containing a sterile larva (*a*); broth culture (control) containing an ordinary larva (*b*). B, Agar slants upon which puparia were placed. The adults emerged and crawled over the surface of the agar. The control tube (*a*) contained a puparium from an ordinary larva; the other tube (*b*) contained a puparium from a sterile larva. C, Sterile larva on sterile bean seedling. The larva attained full growth in nine days and pupated. Imprints of the mouth parts on the agar remained sterile. D, Bean seedling from the tube in C, showing injury to the cotyledons done by the sterile larva

From the results obtained in these experiments it seems necessary to conclude that the presence of bacteria, per se, is not essential to the development and pupation of the larvae of *Hylemyia cilicrura*. It seems permissible to conclude also that in the nutrition of the larvae of this species, the bacteria by their action on the medium sometimes play the rôle of preparing a suitable substratum for growth of the larva.

It seems permissible to conclude also that the substances essential to the growth of these larvae are present in bacteria-free, growing seedlings of beans and some other seeds.

THE OCCURRENCE AND BEHAVIOR OF EMBRYOLESS WHEAT SEEDS[1]

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INTRODUCTION

The occurrence of wheat caryopses entirely devoid of embryos but perfectly normal as to endosperm development has not previously been reported. Harlan and Pope (*11*),[3] working with barley, are the only experimenters who record data concerning a similar phenomenon. They attribute this abnormality to single fertilization. Evidently, according to these workers, only the endosperm fertilization takes place in the development of the embryoless seed. Wheat seeds exhibiting this characteristic have been observed many times by the writer during the course of a study upon the respiration of seeds injured by threshing. It has also been found that such embryo-lacking seeds respire and give evidence of enzyme activity.

OCCURRENCE OF SEEDS WITHOUT EMBRYOS

The rarity of this abnormal development in barley, as reported by Harlan and Pope (*11*), was not found by the writer to be equally apparent in wheat. These workers found only five seeds completely devoid of embryo tissue among many thousands of barley seeds examined, while the writer observed the occurrence of such seeds in each lot of wheat received at the Colorado seed laboratory during the past year. To obtain enough material with which to conduct experiments on respiration and enzyme activity it was necessary to examine minutely large numbers of wheat seeds. In connection with this careful scrutiny of some 150,000 wheat caryopses it was estimated that approximately 0.1 per cent of the seeds of 20 different lots of wheat had developed no embyro. The lots of wheat examined were representative of various varieties of both spring and winter wheats, specifically, Kanred, Turkey Red, Kota, Defiance, and Marquis. It appears from the above that the occurrence of embryo-lacking seeds in wheat is neither uncommon nor limited to one variety.

Embryo-free seeds closely resemble normal seeds, but upon careful examination a deep depression may be noted at the point where embryo development normally occurs. Figures 1, 2, and 3 illustrate the appearance of the embryo-deficient seeds as compared with normal seeds. In Figure 3 it can be readily seen that no embryo development has taken place.

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[1] Received for publication Jan. 30, 1928; issued June, 1928.

[2] The writer wishes to express her grateful appreciation to Anna M. Lute, State seed analyst, for her valuable suggestions and criticisms during the progress of the entire work, and to Dr. L. W. Durrell, professor of botany, Colorado Agricultural College, for his helpful assistance with the manuscript.

[3] Reference is made by number (italic) to "Literature cited," p. 637.

RESPIRATION AND ENZYME ACTIVITY OF EMBRYOLESS SEED

While it is obvious that an embryo-free seed will not grow when subjected to conditions suitable for germination, the absence of the embryo in these abnormal seeds suggests the problem of determining whether or not such seeds exhibit vital processes which accompany germination of the normal seed, such as enzyme activity and respiration. Accordingly a study was made of the catalase activity and the liberation of CO_2 through the respiratory process as a means of determining the activity of those seeds which develop no embryo.

Various investigators have attributed the secretion of enzymes in the Gramineae to three different sources: (1) To the scutellum, (2) to the aleurone layer, and (3) to the amylaceous cells of the endosperm. The secreting power of the epithelial layer of the scutellum in greater or less degree has been noted by practically all investigators, some going so far as to ascribe all secretion to that organ alone. Mann and Harlan (*15*) and Brown and Morris (*6*) from their physiological studies made on barley, and Torrey (*21*), working on germinating maize, conclude that the entire source of diastase and catalase during germination is localized in the epithelial layer of the scutellum. Working with Stoner wheat, Crocker and Harrington (*10*) found that the catalase activity of the embryo is twenty-eight to twenty-nine times greater than that of the endosperm. Bailey and Gurjar (*4*) consider the wheat embryo the seat of respiration. They also state that it is very much richer in enzymes than is the endosperm, and conclude that respiration is decidedly greater in the embryo if not wholly confined to it.

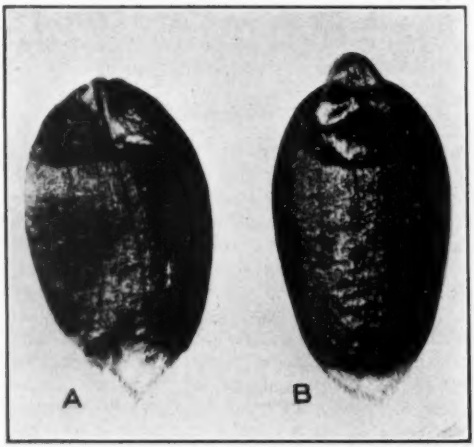

FIG. 1.—A, a wheat seed which has developed without an embryo as compared with B, a normal seed; note the deep indentation at the embryo end of the abnormal seed

From his experiments with whole seeds and excised embryos, Burlakov (*8*) concludes that wheat embryos respire much more vigorously, weight for weight, than do entire seeds, the respiratory ratio being 1 to 16. This conclusion is also supported by observations of Karchevski (*13*) who states that in the wheat embryo the rate of respiration is twelve times greater than in the entire seed. According to Kolkwitz (*14*) the half of a wheat seed containing the embryo respires three times as much CO_2 as the distal half when equal weights of material are compared.

Fewer investigators favor the theories of endosperm secretion which assign vital activity to either the aleurone layer or the starchy endosperm. Bruschi (*7*), experimenting with several species of the Gramineae, maintains that the amylaceous endosperm can digest

itself in varying degrees in the different seeds studied. This self-digestion is made possible because of the fact that much more pro-enzyme exists in the endosperm cells than in the scutellum, and action of this enzyme may cause starch hydrolysis without aid of the embryo. Bruschi's conclusions also indicate that vitality is possessed by the aleurone cells. In like manner Stoward (*19*), working with barley, maize, and castor beans, concludes that the pure endosperm tissue of both barley and maize is capable of evolving CO_2 through the respiratory process. These investigators also attribute a part of the endosperm respiration to the aleurone layer.

All previous work as above noted has been based on studies of seeds which contained both embryo and endosperm, and in order to conduct separate respiration or enzyme tests of these two portions it was necessary to excise the embryos, thus introducing a factor of mechanical injury which might give misleading results.

It has been suggested by Stoward (*19*) that the greater respiratory intensity of isolated embryos may be attributed in part to a wound stimulus received during their removal from the seed. The work of Tashiro (*20*) on wheat seed stimulated by injury bears out this statement, for he shows a marked acceleration in respiration due to injury or wound stimulus. The possibility of error due to injury in separating embryos from their endosperms can not be overlooked. The embryoless seed therefore presents a convenient means of eliminating the effect of stimulus to CO_2 production resulting from wounding. Sufficient embryo-free seeds were found to make possible tests of uninjured seeds containing no embryo structures. To what extent respiration of the normal whole seed is due to the embryo may then be determined by comparison of uninjured embryoless seeds with uninjured normal seeds.[4]

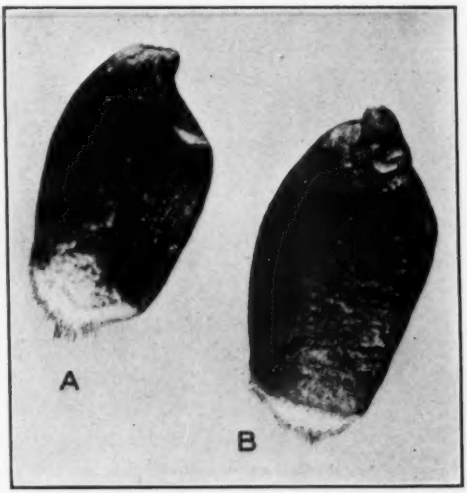

FIG. 2.—Profiles of seeds: A, lacking embryo and B, normal seed. In A the depression due to lack of embryo is very evident

In making these studies a respirometer of simple design was used, consisting of a shell vial 9 cm. deep and 3.5 cm. in diameter, a No. 7 rubber stopper, and a perforated paper cup suspended from the stopper by means of a copper wire. The wire and cup were coated with paraffin in order to prevent any electrolytic effect from the copper wire as well as to render the cup impervious to water.

Five cubic centimeters of CO_2 free saturated barium hydroxide solution is introduced into the shell vial, which is then quickly stop-

[4] THORNTON, B. J. FACTORS CAUSING LOW GERMINATION IN SORGHUM SEED. 1927. [Unpublished, thesis, Colorado Agr. Col.]

pered. A weighed amount of seed, presoaked for 24 hours, in accordance with Braun's (*5*) method of seed treatment and then sterilized for two minutes in a 50 per cent alcoholic solution of mercuric chloride, strength 2:1,000, is then placed in the seed cup. The cup and seed are quickly transferred to the shell by substituting the stopper bearing the cup for the stopper which closes the shell. All respiration tests were run for six days at a constant temperature of 20° C., the optimum recommended for the germination of wheat seeds by the Association of Official Seed Analysts of North America (*22*). At the end of this time the stoppers bearing the seed cups were quickly removed and exchanged for other stoppers. The amount of CO_2 evolved by the seed was determined by titration of the barium hydroxide with N/10 hydrochloric acid and sodium hydroxide. Thymosulphonthalein and tetrabromphenolsulphonthalein were used for indicators as advocated by Harter and Weimer (*12*). Care was taken that all apparatus be kept sterile to insure against the production of CO_2 by microorganisms. In each respiration test made, normal seeds and embryoless seeds from the same lot were used, and all seeds both normal and abnormal were carefully examined with a Hasting triplet 7× lens to reduce to a minimum discrepancies arising from the use of injured seed. In order that the results might be considered comparable, the apparatus and methods described above were used in all of the respiration tests.

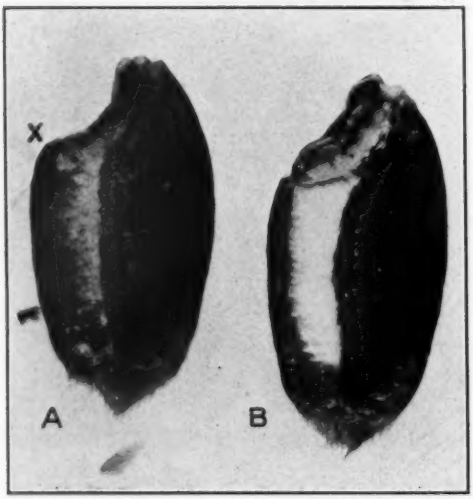

FIG. 3.—Structural differences in longitudinal sections of A, abnormal, and B, normal caryopses of wheat. All embryo tissue is lacking in A, the embryoless seed. The region designated x on this figure corresponds to the region designated x on Figure 4

An average of the results obtained from respiration tests of entire normal wheat seeds shows a liberation of 26.55 mgm. of CO_2 per gram of seed in six days, as compared with 22.09 mgm. of CO_2 evolved per gram of abnormal seed. These data indicate that CO_2 is liberated by embryo-lacking seed under conditions favorable to germination. There being no embryo present, this activity can be attributed to the endosperm only. From the above figures it would appear that the embryo is responsible for approximately one-sixth of the total CO_2 given off by a respiring wheat seed. It is to be remembered, however, that by weight the embryo represents but 3 per cent of the normal seed, and yet, weight for weight, respires about six times as much as the endosperm. The interesting fact is that the experiment was conducted in a way to obviate CO_2 evolution due to injury or to microroganisms, and shows that uninjured endosperm respires and in a measure not to be inferred from previous studies.

It is generally believed that all respiration in plants and animals is coexistent with enzyme action. According to Bailey and Gurjar (*4*), that structure of the seed which is the center of respiration gives evidence of the greatest catalase activity. Crocker and Harrington (*10*), Appleman (*2, 3*), Morinaga (*16*), and Choate (*9*) are among those who have found a rather close correlation between respiratory action and catalase activity. Osterhout (*17*) states that the cessation of the respiratory process is a test for life but that its continuance can not be accepted as a criterion, since CO_2 evolution often continues in an organism which has been killed in certain ways.

The study of the respiration of embryo-free seed therefore was accompanied by tests for the activity of amylase as well as catalase. Small amounts of reducing sugars indicating the presence of amylase were found in the imbibed embryoless seeds after they were kept for six days under conditions suitable for germination.

In testing for catalase, the simplified Bunzel apparatus was employed. Appleman's (*1*) methods were used in conducting the tests. The tissue was ground in a mortar with a small amount of sand and an excess of calcium carbonate. In every case 0.025 gm. of this powdered material was placed in the catalase tube with 5 c. c. of a commercial product, Dioxygen, which had previously been neutralized with N/10 sodium hydroxide. Readings were taken at the end of five minutes.

The results of catalase tests with whole, normal seeds showed an average of 0.17 c. c. of oxygen liberated during a five-minute period for every 0.025 gm. of material used, whereas in the case of embryo-lacking seed 0.12 c. c. of oxygen were evolved per 0.025 gm. of powdered material in the same length of time. These figures indicate that as a result of the catalase activity of a wheat seed 0.12 c. c. of oxygen is liberated by the endosperm while but 0.05 c. c. is evolved by the embryo. This enzyme activity of the endosperm might be inferred from the respiration data given above even though all embryo structures are absent.

STRUCTURE OF EMBRYO-FREE SEED

It is generally believed that enzyme secretion as well as the respiratory process is localized in the epithelial layer of the scutellum. In view of the foregoing data on respiration and enzyme activity, a morphological study of seeds in which the embryo has failed to develop might be expected to show a trace of embryo tissue, or at least some epithelial cells. Many sections of such abnormal seeds were made, but no indication of any embryo development was observed. Figure 4 shows a photomicrograph of a longitudinal section of an abnormal wheat seed; the lack of embryo structures in this seed is quite apparent upon comparison with a section of a normal wheat seed as illustrated by Percival (*18*). No trace of the epithelial layers of the scutellum is evident and all other embryo tissue is absent. The same figure illustrates the junction of the endosperm and the portion normally occupied by the distal end of the embryo. The coat structures are normal, and as in a seed with an embryo, the aleurone cells over the embryo-deficient portion of the seed are greatly diminished in size. The accompanying illustrations exhibit a marked similarity to those of barley figured by Harlan and Pope (*11*).

CONCLUSIONS

Embryoless seeds are of frequent occurrence in wheat. Approximately 0.1 per cent of the lots examined manifest this abnormality. Although the endosperm is normally developed, no trace of embryo tissue is found in microsection.

While these seeds can not grow they do respire when placed under conditions favorable to germination and they also exhibit enzyme activity.

It has been found that wheat seeds devoid of embryos respire 22.09 mgm. of CO_2 per gram of seed in six days, while normal seeds give off 26.55 mgm. of CO_2 in the same time. It is evident from these figures that the endosperm contributes largely to the respiration of whole normal seeds. The embryo constitutes but approximately 3 per cent of the entire seed by weight. On a weight basis,

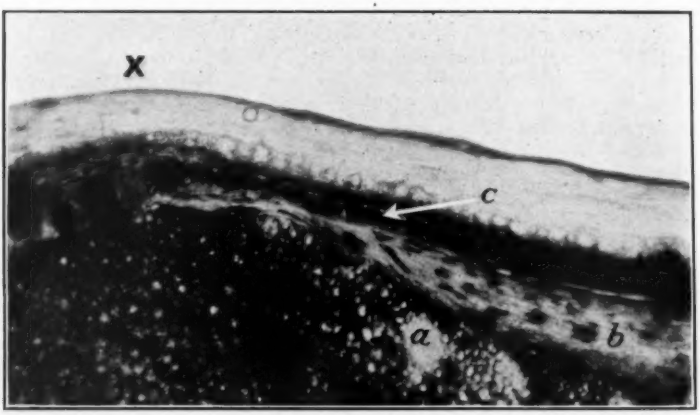

FIG. 4.—Photomicrograph of a section from an abnormal wheat seed taken in the region where the embryo would normally develop: *a*, Amylaceous cells of the endosperm; *b*, collapsed cells apparently of the endosperm tissue; *c*, aleurone layer (note the change in shape of the cells at the point where the aleurone layer of the endosperm joins with that of the embryo cavity; note also that no epithelial layer is present). The region designated x in this figure corresponds with the region designated x in Figure 3

therefore the respiratory activity of the embryo is decidedly greater than that of the endosperm.

Brief tests show some starch conversion in the embryo-lacking seed after six days in the germination chamber.

Further study shows a catalase activity in these seeds equivalent to 0.12 c. c. oxygen production in five minutes as compared to 0.17 c. c. oxygen produced in the same length of time by equal weights of normal seeds.

Various investigators have studied the respiration of the embryo in its relation to the entire seed, but always after excising the embryo. The occurrence of embryo-lacking seeds offers a solution to the questioned effect of mechanical injury in studying respiration of the embryo as compared to the endosperm.

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A SIMPLE METHOD FOR LIFE-HISTORY STUDIES OF ROOT-FEEDING ARTHROPODS [1]

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INTRODUCTION

Detailed studies of the life histories of insects which spend all or part of their life cycle feeding upon the underground parts of plants have been exceedingly difficult because of the inaccessibility of the specimens to ready observation. The eggs of most insects which pass their immature stages underground are usually deposited in the soil on or near their host and are generally difficult to find. After the eggs have hatched the immature forms are not readily located and a search for them, no matter how carefully conducted, usually results in the accidental destruction of a large percentage of the specimens.

These difficulties were constantly encountered in an investigation involving a study of the larvae of the striped cucumber beetle (*Diabrotica vittata* Fab.). In these experiments it was necessary to use large numbers of the larvae and to be able to determine their condition at any time, as well as to be assured of a sufficient supply of specimens at any required stage in the life cycle. It was impossible to obtain these conditions when the beetles were permitted to reproduce in the usual manner.

After some experimenting, a method for the collection and incubation of eggs, the maturing of larvae, and the production of adults, all under immediately accessible conditions, was perfected and found to be very advantageous for the purpose of the investigation. Some additional study, in which various other insects and arthropods were used, has demonstrated that the method is easily adaptable to the investigation of the life histories of most of the forms which spend at least a part of their life in the soil. This article, however, will deal only with the method as it was employed in the study of the development of the striped cucumber beetle.

CAGE FOR COLLECTION OF EGGS

Cylindrical cages of 12-mesh wire screen cloth 10 cm. long and 6 cm. in diameter were used for confining male and female beetles. These were made by soldering to one end of each cylinder the bottom cut off of a tin salve box; the upper part of the box, over which the cover fitted, was soldered to the other end of the cylinder. (Fig. 1.) The cover of the salve box was used to close the cage. The cages were laid upon moist blotting paper and pressed into a quantity of soft soil heaped up in pie tins. Green blotting paper to contrast with the orange color of the eggs was used. By placing the cages in

[1] Received for publication Feb. 20, 1928; issued June, 1928.

pie tins they could be readily moved to any desired location. Food could be easily placed in the cages by simply removing the salve-box cover from the end of the cage.

The beetles oviposit through the wire screen cloth upon the blotting paper. The eggs are then exposed by lifting the cage from the blotting paper. They adhere to the moist blotting paper but are easily removed when the paper dries. The drying should be done slowly and carefully in order to prevent the collapse of the eggs. It is feasible, but not so expeditious, to dispense with the blotting paper, since the screen walls of the cage will restrain the beetles from placing their eggs in the soil. In such cases the eggs will be found on the soil adjacent to the walls of the cage. By varying the number of females in a cage, it is possible to determine and collect the number of eggs laid by one or by a number of females.

FIG. 1.—Oviposition cage for the striped cucumber beetle. Beetles confined in the cage oviposit through the screen wire on blotting paper laid over the surface of the soil

CAGE ARRANGEMENT FOR INCUBATION OF EGGS

Cells made from short pieces of glass tubing, one end being stopped with plaster of Paris, were used for the incubation of eggs. (Fig. 2.) Glass cells ranging in height from 1 cm. to 3 cm. and in diameter from 1.3 cm. to 10 cm. were tested. Cells 3 cm. in diameter and 2.3 cm. in height, however, gave the best results. Various thicknesses of plaster of Paris in the cells were tested, and a thickness of 0.3 cm. proved to be the most satisfactory. A convenient method for obtaining uniformity of depth of the plaster of Paris in the cell consists of first mixing the plaster with water to the consistency of fresh cream and then pouring the mixture to a depth of 0.3 cm. into paraffined pie tins. The sections of glass tubing are then placed on end in the mixture. Melting the paraffin after the plaster of Paris has set releases the pie tin, and the plaster of Paris can be removed readily

from each cell. It is necessary that all paraffin adhering to the bottom of the cells be removed. If it is allowed to remain, it will hinder the passage of the water into the cells. To provide a ready method of maintaining a fairly constant moisture content in the cells, they are placed open end upward in soil in pie tins to a depth equal to that of the height of the plaster of Paris in the cells. (Fig. 2.) A number of the cells may be placed in the same pie tin, but care should be taken to see that there is a small area of soil space between each cell. The moisture supply is maintained by adding water at intervals to the soil in the pans. It should be only sufficient to keep the surface of the plaster moist. The presence of free water on the

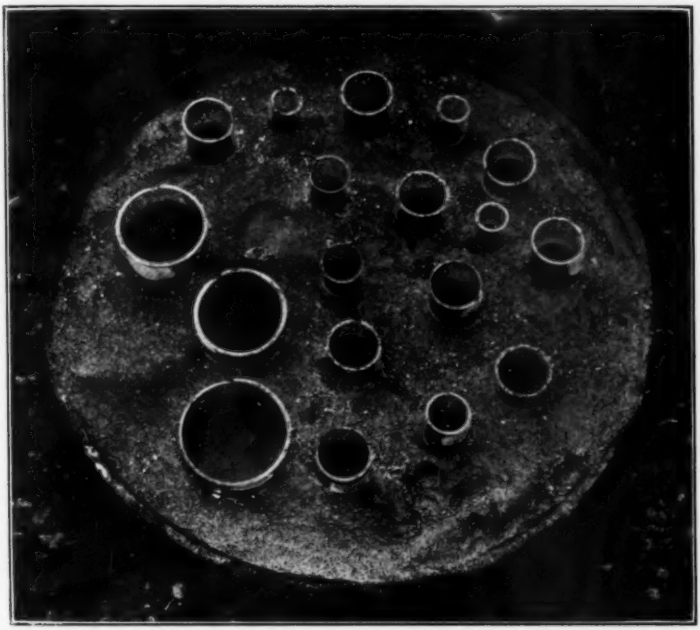

FIG. 2.—Incubation cells used in studies on the striped cucumber beetle. Sections of glass tubing filled to a depth of 3 mm. with plaster of Paris, pressed into moist soil, and the soil between the cells covered with sand

plaster of Paris will cause the death of the embryos. Covering the soil with sand to a depth of about 1 cm. between the incubation cells will aid materially in reducing soil evaporation. Placing the incubation cells in pie tins renders them easily portable.

The larvae and eggs are made easily visible by painting the surface of the plaster with black india ink.

Under this method of treatment the eggs were completely accessible throughout the incubation period. The viability of the eggs as well as the exact moment of emergence can be determined, and the behavior of the larvae upon hatching and the effect of temperature and humidity upon the eggs can be noted. Many eggs, the number

depending upon size, can be placed in one cell for incubation. It is necessary only that the eggs be in contact with the moist plaster of Paris.

CAGE ARRANGEMENT FOR REARING LARVAE

Petri dishes containing plaster of Paris were used in rearing the larvae. (Fig. 3.) These were prepared as follows: Plaster of Paris mixed with water to the consistency of fresh cream was poured into the dishes to the depth of about 6 mm. and permitted to set. Squash plants in the seedling stage were removed from the soil in which they had grown, practically all of the soil washed from the roots, and the plants placed in the Petri dishes with the roots spread out upon the

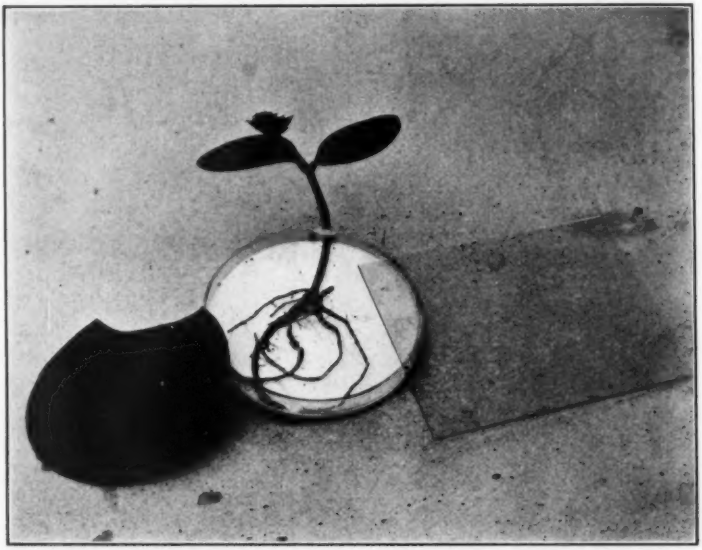

FIG. 3.—Details of Petri-dish rearing cage for larvae of the striped cucumber beetle. The blotting paper shown at the left was laid over the roots and the whole saturated with the nutrient soluton. The glass cover shown at the right was then placed over the dish

plaster of Paris and the leaves extending outward over the sides of the dishes. Pieces of blotting paper cut to fit closely to the sides of the dishes, except where the stem of the plant extended from the dish, were then laid over the roots and the whole saturated with a nutrient solution. To assist in the retention of the larvae and to avoid rapid evaporation of the solution, pieces of glass were laid over the dishes, a small opening being left at the plant stem to permit aeration. It is quite feasible to use larger Petri dishes for larger arthropods and larger plants. A flat dish with a lip could be used conveniently, since the lip would provide an opening for the stem of the plant. The part of the opening not occupied by the stem could be filled with raw cotton.

FORMULA FOR NUTRIENT SOLUTION

The nutrient solution used was one suggested by W. E. Tottingham, professor of agricultural chemistry at the University of Wisconsin, and was of the following formula:

	Grams		Grams
Sodium nitrate	2.0	Calcium sulphate (hydrated)	0.2
Disodium phosphate (hydrated)	.2	Magnesium sulphate (hydrated)	.2
Monosodium phosphate (hydrated)	.1	Ferric citrate (made by heating a 1 per cent solution, 1 gm. to 100 c. c. of water. Use 5 c. c. per liter)	.05
Potassium chloride	.4		
Water to make 1 liter.			

This is a general-purpose formula. Legumes as well as cucurbits and grasses grew well in the Petri dishes when a solution made by this formula was used.

MANIPULATION OF LARVAL CAGES

When the solution is used, it is necessary that an excess be avoided, since free liquid about the roots will cause the death of both plants and larvae. The insect and plant thrive best when the plaster of Paris and the blotting paper are merely moist. The quantity of solution necessary to maintain this condition varies with the temperature and humidity of the surrounding atmosphere, but it can be easily determined by noting the condition of the blotting paper. The addition of about 4 c. c. of the solution to each dish daily was usually sufficient.

TRANSPLANTING PLANTS

In these experiments plants of Hubbard squash were used because of their large size and strong root system. To obtain the best results it was necessary to germinate the plants in soil and to remove them to the Petri dishes while they were still in the cotyledon stage and before the fibrous roots were formed. In experiments with cucurbits and legumes it was found that the plants were not able to utilize the solution except through the water roots. Old plants which had lost their water roots either died or remained wilted until new water roots were formed.

TRANSFERRING LARVAE

The newly emerged larvae are easily injured and should be handled with great care. It was found that they could be transferred without injury from the incubation cells to the Petri dishes by barely touching them with the flattened end of a piece of wire which had been slightly moistened with water. The larvae were immediately held to the wire by the surface tension of the water; this evaporated quickly and released them at any desired place in the Petri dish at which the wire was held.

OBSERVING LARVAE

To observe the larvae at any time it is necessary only to remove the cover glass and blotting paper. This is one of the greatest advantages of the method since the insects are always readily accessible. In addition, a great many of them may be reared in a small space. (Fig. 4.)

CONTAMINATION OF THE CAGES WITH GREEN ALGAE

Green algae grew readily in the nutrient solution and caused some trouble. When the alga becomes abundant, it forms a coating over the paper and the plaster, inhibiting the free absorption of the solution. When this condition exists, the plants do not grow well, and therefore care should be taken to prevent the algae from contaminating the stock solution or the Petri dishes. Autoclaving the solution and changing the blotting papers occasionally will serve to hold the algae in check. Petri dishes used as cages may be salvaged for further use by subjecting them to dry heat at a temperature of about 500° F. for one-half hour. The plaster of Paris contracts under this treatment and is readily removed.

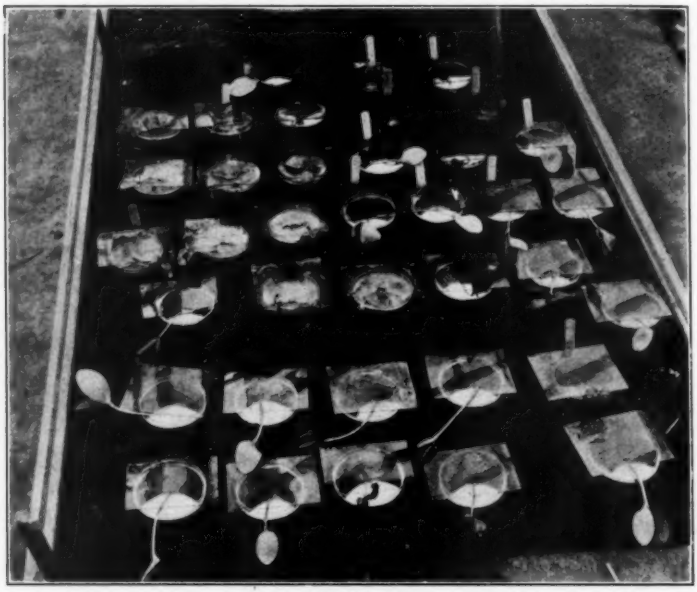

FIG. 4.—Series of rearing cages used in life-history studies of the striped cucumber beetle. This series occupied about 5 linear feet of a greenhouse bench and was sufficient for rearing about 300 larvae

CAGES FOR PUPATION

When the larvae reach the prepupal stage, as indicated by restlessness and cessation of feeding, they can be removed to small pasteboard pill boxes containing a small quantity of moist soil.

LENGTH OF LIFE CYCLE

The conditions imposed by the method herein described may accelerate or retard the development of some species of arthropods, and for accurate life-history studies it would be well to run a check by rearing some specimens under natural conditions in soil. It was found that the striped cucumber beetle completed a life cycle, under the conditions imposed by this method, in an average of 34 days.

Beetles permitted to reproduce on potted squash plants required an average of 37 days to complete the life cycle. The time required for the completion of each stage under each method was almost exactly proportional to the average total time required under each method. This condition may not hold with all other arthropods, and the duration of stages in the life history of those reared in plaster of Paris cages and of those reared under natural conditions should be considered comparable only where tests have shown them to be comparable.

ADVANTAGES OF THE PLASTER OF PARIS CAGES

1. Individuals or groups are immediately accessible for any purpose throughout their life cycle.
2. Phytopathological studies involving arthropods are greatly facilitated.
3. Individuals or groups may be easily segregated throughout the life cycle. Pedigreed specimens may be reared if desired.

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A STUDY OF THE APPARENT VISCOSITY OF MILK AS INFLUENCED BY SOME PHYSICAL FACTORS[1]

By GEORGE MONROE BATEMAN[2] and PAUL FRANCIS SHARP, *Department of Dairy Industry, New York State College of Agriculture, Cornell University*

INTRODUCTION

For many years numerous studies of the viscosity of whole milk and cream have been carried out. The methods used by most of the investigators have been very empirical. No careful investigation of the viscosity of milk and cream at various rates of shear has been reported in the literature.

A great variety of conditions and methods were used to determine the viscosity of milk. This makes it impossible to compare the work of one experimenter with that of another, except in a general way, since one does not know whether or not their results are independent of the dimensions and peculiarities of the apparatus they used. The correction for kinetic energy or inertia has been ignored. Some investigators have made a comparison of the relative rates of flow of water and milk by means of an ordinary pipette, and have apparently assumed that this method gave a measure of viscous flow, since they have designated their results "relative viscosity." In most of the work the standard of comparison was water, and frequently no attempt was made to run the determinations at exactly the calibration temperature. The importance of careful temperature regulation is paramount when it is realized that the fluidity of water changes from 1 to 3 per cent for a change of 1° C. Most of the investigations have been carried out at widely varying temperatures, which increases the difficulty of the problem of weighing the relative value of the results. Dahlberg and Hening (*13*)[3] worked in a refrigerator where the temperature was kept between 3° and 4° C. Kobler (*20*) carried out most of his experiments within a temperature range of 15° to 18° C., while Taylor (*30*) worked at 20° C. Cavazzani (*12*) studied the viscosity of milk at 37°. A number of workers have ignored the difference in density between the calibration liquid, water, and the milk or cream whose viscosity was being determined. This would be a source of error in an Ostwald viscometer where the driving force is entirely due to the hydrostatic head of the contained liquid. The omission of such data as fat content, total solids, previous history of the sample, etc., also complicates the difficulty of making a quantitative comparison and estimation of the published work on the viscosity of milk and cream.

Another subject which has been largely ignored in the past is, how the apparent viscosity of skim milk is affected by different physical factors. In almost all the work done only the viscosity of whole

[1] Received for publication Dec. 23, 1927; issued June, 1928.

[2] Abstracted from a portion of a thesis presented by George Monroe Bateman to the graduate school of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1927.

[3] Reference is made by number (italic) to "Literature Cited," p. 672.

milk and the factors that influenced it have been considered. Very little study has been made of skim milk as a separate phase in which changes are taking place along with those (clumping and creaming) in the fat phase. Consequently, very little is known concerning the changes in whole milk which are actually due to the alterations taking place in the skim-milk phase.

No investigator of the viscosity of milk and cream has taken the later concepts of plastic and viscous flow into consideration in his work.

The conditions of flow which a liquid must fulfill when a given volume is forced through a capillary using various pressures to produce the flow, in order that it may be said to follow Poiseuille's law, are as follows:

$$\eta = Pt \text{ (constant)} \quad [4] \tag{1}$$

where η is the viscosity coefficient, P the pressure, and t the time of flow. This equation shows that the viscosity of a liquid which follows Poiseuille's law is independent of the pressure producing the flow. The extent to which the flow of milk through a capillary is in agreement with this relationship has never been accurately determined.

Since the conditions under which equation (1) holds experimentally are inconvenient for investigational work, the equation has been modified by correction factors so that the capillary need not be so small and so that high pressures can be used. Bingham and White (*8*) have used the equation in the following form:

$$\eta = \frac{\pi g r^4 Pt}{8v(l+\lambda)} - \frac{mv\rho}{8\pi t(l+\lambda)} \tag{2}$$

where η = viscosity coefficient.
g = gravitational constant.
r = radius of capillary in centimeters.
P = pressure in grams per square centimeter.
t = time of flow in seconds.
v = volume of flow in cubic centimeters.
l = length of capillary in centimeters.
m = constant whose value was taken as 1.12.
ρ = density of liquid.
λ = a correction to be added to the length of the capillary to correct for the modification of the stream lines at the terminals of the capillary, which cause a loss of pressure in overcoming friction.

The term involving m is generally called the kinetic energy correction, and, as will be seen from equation (2), it is a subtractive correction to be applied to Pt to cover a loss of head or pressure rising from the imparting of kinetic energy to the liquid. This correction is usually very small and approaches zero in the case of long, narrow capillaries, or in cases where the value of the pressure, P, is very small.

[4] This equation holds experimentally only for very long, narrow capillaries or for very small pressures.

EXPERIMENTAL

APPARATUS AND GENERAL PROCEDURE

This work was undertaken, first, to make a systematic study of the viscosity of skim milk under varying conditions of treatment; second, to see if some of the changes which take place in the viscosity of whole milk are due to changes associated with the fat or to changes in the skim-milk phase; and third, to investigate the rate of flow of milk through capillary tubes at varying pressures. Practically no work has been done on these phases of the subject.

The methods outlined below are general. The special procedure followed in each phase of the experimental work will be presented, together with the resultant data.

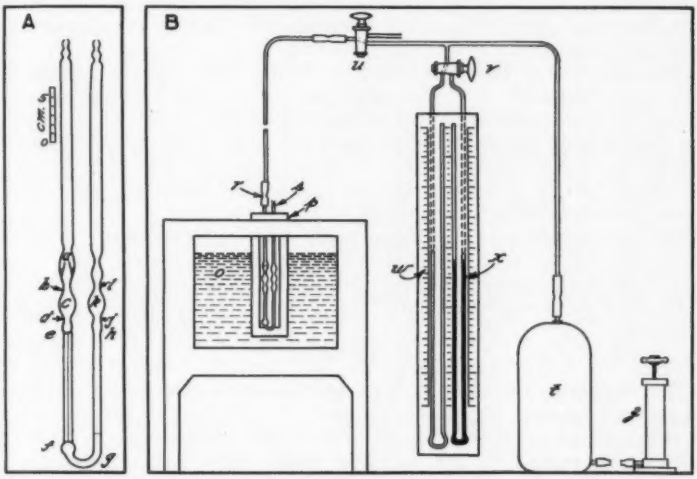

FIG. 1.—A, Important features of the Bingham viscometer; B, apparatus used to apply variable pressure to the viscometer

MILK USED

The milk used in the experiments was obtained from the Cornell University dairy herd. It was filtered through cotton into sterile bottles, and immediately after milking it was cooled to 0° C. to prevent, as far as possible, contamination or decomposition. When skim milk was desired, the fat was removed by centrifuging the properly cooled whole milk in 250 c. c. bottles at about 2,500 revolutions per minute for 30 minutes. The skim milk was carefully removed, filtered into sterile storage bottles, and cooled to about 0° C. In all cases dust contamination was guarded against.

Since most of the milk used in the experiments was obtained under conditions that would insure a low bacterial content and freedom from dirt, no attempt was made to get representative samples. The milk was taken from single cows at various stages of milking. Hence in many cases the ratio between total solids and milk fat was not always representative of normal whole milk.

The fat content was determined according to the Roese-Gottlieb method as modified by Mojonnier (Mojonnier and Troy (*22*)). The milk solids determinations were also carried out with the Mojonnier milk solids testing apparatus.

Apparatus

Figure 1, A, gives the details of the Bingham (*6*) viscometer, and Figure 1, B, shows a view of the whole set-up of the apparatus used. *t* represents a galvanized tank of about 25 liters capacity, with an automobile tire valve soldered to it to serve as an intake valve. Air was compressed into the tank by means of a hand pump *z*. Glass tubing connected the tank *t* to the manometers (*x* mercury and *w* water), and to the viscometer at *r*. At low pressures the water manometer, *w*, was used, while at higher ones the mercury manometer, *x*, could be utilized by turning the three-way stopcock, *v*, halfway around.

The viscometer was connected to the pressure line by means of a three-way stopcock, *u*. This stopcock when turned in one direction connected the viscometer to the laboratory air, and when turned in the other connected it to the pressure line. The viscometer was held rigidly in a solid wooden frame, *p*, which fitted in a slot in the water bath, *o*, so that it could be replaced in the same position after removal from the water bath for cleaning.

The temperature of the water bath was kept constant $\pm 0.01°$ C. by means of an automatic mercury thermo regulator. All determinations were carried out at 25° C. A Beckmann thermometer was immersed in the water bath to check the temperature at frequent intervals.

Calibration

The Bingham viscometer used in this work was purchased from Eimer and Amend, and the capillary was specified as 500, according to the requirements set by Bingham (*6*). With such a capillary fluidities from 0.5 to 500 can be measured. This viscometer, if properly made, has all the desirable features of the Ostwald viscometer, and practically none of its objectionable ones. From the diagram (fig. 1, A) it will be noted that the viscometer is constructed so as to reduce the effect of the hydrostatic pressure of the contained liquid to zero. Each arm of the instrument is made as nearly as possible similar to the other in shape and height, and the bulbs have the same volume. The value of the density of the contained liquid need not be known with considerable accuracy. A trap is provided in one arm to make it possible to work with exactly the same volume of liquid at all temperatures.

With the Bingham viscometer the force driving the liquid through the capillary is entirely due to the pressure that is being exerted on the system, providing the viscometer is constructed so that the hydrostatic head of the contained liquid is equal to zero.

Inasmuch as it would be too difficult and laborious to construct a viscometer for ordinary work where all of the dimensions could be accurately determined by direct measurement, it is a common practice to calibrate the instrument with some suitable standard liquid. For a given instrument it will be noted that certain of the factors in equation (2) remain constant in all of the determinations and that these may be presented by C and C'. Hence

$$C=\frac{\pi g r^4}{8lv} \tag{3}$$

and for the kinetic energy correction factor,

$$C'=\frac{mv}{8l\pi} \tag{4}$$

Equation (2) now becomes:

$$\eta=CPt-C'\rho/t \tag{5}$$

where C and C' are constants, which may be determined.

In this work the value of C' was found by direct measurement. The volume, v, of the bulb, c (fig. 1, A), between the marks b and d was found to be equal to 4.0 c. c. and the length of the capillary 10 cm. Hence

$$C'=\frac{1.12\times 4.0}{8\times 3.1416\times 10}=0.01784$$

To determine the viscometer constant C, it was first necessary to solve equation (5), which gives:

$$C=\frac{\eta t+C'\rho}{Pt^2} \tag{6}$$

A number of viscosity runs were made with dust-free, freshly distilled water at 25° C. to determine the time of flow at known varying pressures for each limb of the viscometer. The mean value of the viscometer constant was found to be equal to 1.319×10^{-7}.

For the viscometer used in this work, equation (5) now becomes:

$$\eta=1.319\times 10^{-7}Pt-0.01784\rho/t \tag{7}$$

where η is the viscosity in centipoise.

The flow periods for each arm were found to be approximately the same, since no variation was greater than one-fifth of a second. Hence the correction for the hydrostatic pressure of the liquid in the viscometer was unnecessary with this instrument.

In the course of these experiments we tried to maintain a constant room temperature. The effect of slight variations in the temperature of the room and the corresponding effect on the pressure in the tank was one of the greatest sources of error with which the authors had to contend. This error could have been greatly reduced by placing the pressure tank in the water bath. The change in pressure due to slight changes in temperature during each determination was usually so small that it could not be detected on the manometers. Also it was impossible to measure time periods with greater accuracy than one-fifth of a second with an ordinary stop watch. In order to minimize the above sources of error as much as possible, the efflux periods throughout these experiments were largely maintained between 100 and 1,000 seconds. The stop watch was always kept wound to about the same tension, and at frequent intervals it was checked for accuracy by means of a standard chronometer.

THE INFLUENCE OF PRESSURE, AS A VARIANT, ON THE APPARENT VISCOSITY OF SKIM MILK, SKIM COLOSTRUM, RAW, PASTEURIZED, AND HOMOGENIZED WHOLE MILK, AND EVAPORATED SKIM MILK

It is rather surprising that most of the investigators have overlooked the effect of the rate of shear on the viscosity of milk and cream.

Galdi (*15*) attempted to determine the viscosity of a large number of liquids, including milk, by means of a Hirsch-Beck apparatus. His apparatus really consisted of a modified Ostwald viscometer connected to a source of pressure. He determined the relative viscosity of milk at a number of pressures, but his data show comparatively large inconsistent variations. His results vary between 1.57 and 1.67 and are of little value in showing the effect of the rate of shear on the viscosity of milk.

The only other reference covering the relation between the viscosity coefficient of milk and the pressure that could be found in the literature was a statement by Rothlin (*26*) that mixtures of emulsoids and suspensions such as milk, defibrinated and normal blood, show deviations from Poiseuille's law at low rates of shear. Rothlin (*25*) presented no results on milk, however, even in his more extensive paper.

If Rothlin's observations are correct the question as to absolute values for the viscosity of milk arises. If a physical "constant" shows variation with the conditions it is being subjected to when determined, it is obvious that these conditions should be specified. This is especially true where changes in the structure of the constituents caused by various kinds of treatment are being considered.

These series of experiments constitute a study of the influence of varying the rate of shear on the apparent viscosity of skim milk, skim colostrum, raw, Pasteurized, and homogenized whole milk, and evaporated skim milk. The viscometer was filled accurately with a definite volume of milk, in such a manner that the aliquot of the test milk was not passed through the capillary in the filling process. The viscometer was cleaned and dried for each individual determination.

The data which follow were selected from a number of experiments as being typical of the effect of the rate of shear on the viscosity of milk.

TABLE 1.—*Effect of varying pressure on the viscosity of skim milk*

Sample No.	Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
1	1,146.3	96.4	1.438
	982.0	112.6	1.442
	681.6	162.7	1.450
	480.3	231.4	1.458
	278.1	401.4	1.468
	150.1	743.4	1.470
2	981.2	109.0	1.393
	814.8	131.4	1.398
	682.2	157.2	1.403
	480.3	223.0	1.405
	278.1	385.0	1.408
3	981.2	117.2	1.500
	814.8	140.0	1.491
	681.6	168.8	1.506
	480.9	238.6	1.507
	278.1	415.6	1.519
	143.1	808.6	1.516
	71.6	1,612.8	1.516
4	981.7	113.3	1.452
	815.7	136.8	1.458
	682.4	163.9	1.464
	481.7	232.3	1.468
	278.7	402.2	1.474
	147.9	760.4	1.481
	71.9	1,579.2	1.496

TABLE 2.—*Effect of varying pressure on the viscosity of fresh skim colostrum*

Number of days after parturition	Pressure gm. per sq. cm.	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
4	1,306.14	104.4	1.781
	979.7	139.3	1.787
	679.3	201.6	1.797
	275.3	498.4	1.806
	132.21	1,051.2	1.831

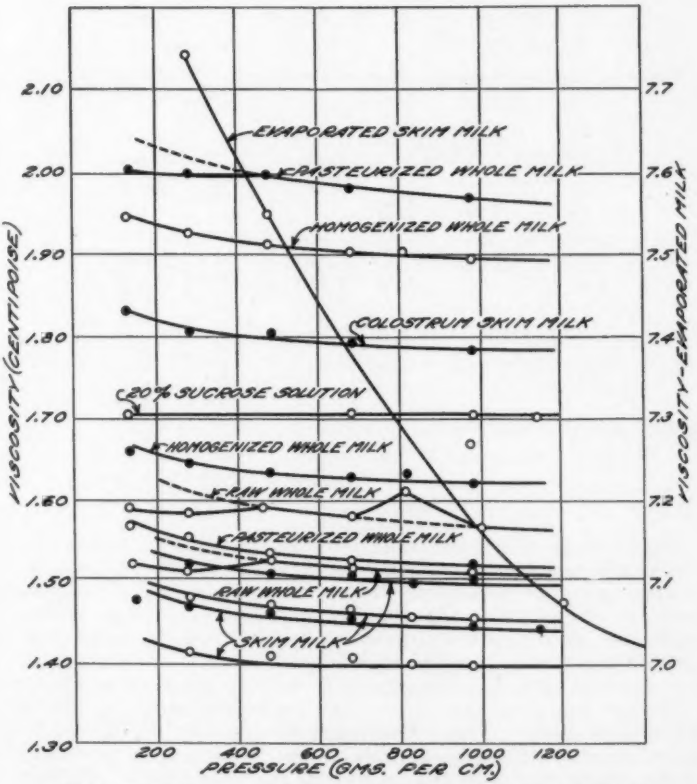

FIG. 2.—Decrease in viscosity of various kinds of milk as shearing force is increased

The viscosity of skim milk and skim colostrum at varying pressures was studied first. The results are given in Tables 1 and 2, and graphically in Figure 2. From these data it can be seen that the viscosity coefficient of skim milk is not constant but varies with the pressure. When the pressure is increased the apparent viscosity is slightly decreased. Skim colostrum (four days after parturition) also gave pressure-viscosity curves which resembled those of skim milk.

TABLE 3.—*Effect of varying pressure on the viscosity of raw whole milk*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
4	3.58	12.86	1,306.9	91.9	1.548
			978.3	122.7	1.567
			812.1	151.9	1.613
			679.4	177.7	1.581
			477.5	254.0	1.591
			275.9	437.0	1.587
			131.8	917.8	1.593

TABLE 4.—*Effect of varying pressure on the viscosity of Pasteurized whole milk*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
8	4.75	14.18	980.4	153.2	1.970
			680.1	221.7	1.981
			481.5	314.9	1.999
			277.2	548.5	2.001
			145.85	1,051.9	2.023
6	3.83	12.82	1,306.9	89.6	1.523
			979.7	118.2	1.511
			678.8	170.4	1.514
			478.8	242.6	1.524
			275.9	418.2	1.514
			138.0	834.6	1.522
1	----------	----------	1,308.5	88.4	1.511
			980.6	118.7	1.517
			680.9	171.2	1.526
			478.9	244.2	1.535
			276.7	427.4	1.555
			138.2	863.0	1.571

Experiments with whole milk are given in Tables 3 and 4, and are graphically represented in Figure 2. Much difficulty was experienced in attempting to determine the apparent viscosity of raw or Pasteurized whole milk. At the lower pressures it was practically impossible to obtain concordant results. It will be noted that the curves for raw and Pasteurized whole milk shown in Figure 2 are not all consistent, and that the apparent viscosity values at low pressures are much less than the theoretical (extrapolated) ones, shown by the broken lines. These discrepancies are due to the fact that at low pressures the time periods are sufficiently long to allow part of the cream to rise to the surface in the viscometer. This means that as the time of flow increases, more of the butter fat will have a chance to rise, and the composition of the milk flowing through the capillary will approach that of skim milk. Hence its apparent viscosity will tend to decrease with the longer time periods.

The results obtained with homogenized whole milk appear to support the above conclusion. In homogenized whole milk the fat globules are not clumped and are broken up into sufficiently finely

divided particles so that they remain suspended throughout the milk. With such a system a viscosity-pressure curve that resembles those of skim milk and skim colostrum can be expected. The results obtained with homogenized whole milk are given in Table 5, and are plotted as curves in Figure 2. The viscosity values obtained with homogenized whole milk were very consistent and reproducible.

TABLE 5.—*Effect of varying pressure on the viscosity of homogenized whole milk*

Number of days aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
1	2.94	11.64	1,307.8	94.8	1.616
			980.6	126.6	1.623
			815.6	152.2	1.626
			680.9	182.5	1.629
			480.3	259.3	1.636
			278.1	450.0	1.647
			136.4	920.0	1.659
1	4.63	14.08	1,306.9	110.6	1.892
			979.7	147.3	1.892
			812.7	178.4	1.902
			679.4	213.2	1.901
			479.7	303.7	1.915
			276.6	529.5	1.928
			134.46	1,059.6	1.947

The sample of evaporated skim milk studied in this series of experiments, showed the greatest variation of viscosity with pressure. A glance at Figure 2 and Table 6 makes this fact evident.

TABLE 6.—*Effect of varying pressure on the viscosity of evaporated skim milk*

Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
Fat	Total solids			
0.72	25.63	1,466.2	366.8	7.089
		980.4	562.0	7.252
		814.2	671.2	7.205
		480.3	1,186.2	7.513
		276.0	2,127.2	7.744

In order to make sure that the value of the capillary constant C was correct, and that the results, as obtained, were not due to some peculiarity of the apparatus, a set of runs were performed on a 20 per cent pure sucrose solution. According to Bingham and Jackson (7) this solution should have a viscosity of 1.704 centipoise at 25° C. As will be noted by referring to the data in Table 7 and Figure 2, the viscosity of the solution at all of the pressures was constant and averaged 1.704 centipoise. This shows that the apparatus was apparently not the cause of the variation in the viscosity of milk with pressure.

TABLE 7.—*Effect of varying pressure on the viscosity of a 20 per cent sucrose solution* [1]

Pressure (gms. per sq. cm.).	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
1,144.9	113.8	1.703
980.6	132.9	1.704
680.9	191.0	1.704
124.7	1,037.2	1.704

[1] Viscosity according to Bingham and Jackson (7) = 1.704.

If milk were a truly viscous liquid, it should give a constant viscosity value with varying pressures, since according to Poiseuille's law the product of the pressure and time for a given set of conditions should equal a constant. This means that the pressure-viscosity curve should be a straight line parallel to the pressure axis. Since milk does not fit the requirements of the law of Poiseuille, but shows a slight variation with pressure, it can not be called a truly viscous liquid. No term which can be used to designate the flow of a liquid which deviates slightly from Poiseuille's law has come into common use. The terms "apparently viscous" and "plastico-viscous" have been used to designate flow of this type. Inasmuch as the viscosity of milk is not independent of the rate of flow, it can be readily seen that all of the conditions of measurement must be taken into consideration. Hatschek (*17*) says: "If the viscosity of a given sol varies with the shear gradient, single measurements at arbitrary and unknown shear gradients have no theoretical value, least of all as a foundation for hypotheses regarding structure."

It has already been noted that the viscosity of milk is a function of the pressure and decreases as the pressure increases. The assumption which Hatschek and Jane (*18*) make to account for this variation is that each particle must be surrounded by an adsorbed layer of liquid, which increases its effective volume and moves with it. This layer is assumed to be very labile, and it is more and more reduced in thickness as the rate of flow increases. The thicker the adsorbed layer the greater will be the viscosity. It will be seen that the decrease of viscosity with increased rate of flow will be related to the amount of this adsorbed film displaced, and that at pressures sufficiently high, the viscosity should approach a constant value.

Hess (*19*) has also offered an explanation for the decrease in viscosity of suspensions with an increase in shearing force. He considered that the suspended particles, due to their size, obstructed the normal telescopic flow of the dispersion medium, causing "dead" spaces immediately behind and ahead of the particles. These so-called dead spaces removed a part of the liquid from the normal telescopic flow of the dispersion medium and consequently increased the effective volume of the particle, and at the same time decreased the effective volume of the dispersion medium. He believed that as the shearing force increased the telescopic layers displaced more of the liquid in the dead spaces, and consequently the viscosity decreased as the shearing force increased.

THE EFFECT OF REPEATEDLY FORCING MILK THROUGH A CAPILLARY TUBE AT CONSTANT PRESSURE

Kobler (*20*) studied the effect of repeatedly running milk through a capillary, and of agitation, on the relative viscosity of whole milk. He forced it through a capillary 40 times, and found that the viscosity was materially diminished. For example his first reading was 1.77, his tenth decreased to 1.70, his twentieth to 1.65, while his values from the thirtieth to the fortieth remained at 1.60. However, after the milk was allowed to stand for 14 hours the viscosity of the sample was found to be 1.70. He also showed that he could diminish the viscosity of normal milk from 1.83 to 1.67 by 20 minutes of agitation, and that if he allowed it to stand for 14 hours the viscosity would return almost to the normal value, if the shaking had not been too severe. Kobler assumed that the decrease was due to the breaking up of a rigid structure in the milk which was capable of forming again on standing, provided the agitation had not been too severe. He made no attempt to explain the actual mechanism of this structure. It is probable that this structure, postulated by Kobler, was really a clumping of the fat globules, and that the decrease in viscosity noted was largely brought about by breaking up these clumps.

This set of experiments constitute a study of the effect of repeatedly running skim milk, skim colostrum, raw and homogenized whole milk through a capillary. The effect of protein and fat grouping as factors influencing the constancy of the viscosity was especially considered.

Much difficulty was experienced with raw whole milk, since the cream had a tendency to rise slowly and stick to the sides of the viscometer reservoirs during the progress of experiments. Churning also had to be guarded against. With homogenized raw milk there was no indication of the fat sticking to the viscometer.

The raw whole milk was secured immediately after milking and cooled to 4° C. It was aged at this temperature for a number of hours to allow the fat globules to clump thoroughly. The milk was next gently mixed by pouring it from one vessel to another. A sample was placed in the water bath and allowed to come to a temperature of 25° C. The milk was again mixed very gently; a portion was added to the viscometer, and continuously forced back and forth through the capillary at constant pressure. At the same time, another part of the same sample was examined under the microscope to note the state of aggregation of the fat globules. The milk was introduced into trenches in a blood-counting cell. A cover glass had been previously cemented over the trenches by means of a mixture of vaseline and paraffin, and after the milk was introduced into the trenches the open ends were sealed with the same paraffin mixture. This gave a thin uniform layer of milk. The microscope was adjusted so that the trenches could be examined in a perpendicular position. The degree of clumping of the fat globules and their tendency to rise either as individuals or as aggregates could be followed very closely. This procedure was repeated with the milk after it had been repeatedly forced through the viscometer capillary.

The milk which was to be homogenized was first carefully heated to 50° C., and then forced through the homogenizer at a pressure of over 4,000 pounds. It was immediately cooled to about 4° C. and kept at this temperature until used. Throughout this work all of the homogenized milk was subjected to the above treatment unless otherwise stated.

The results of this series of experiments are summarized in Tables 8 to 11.

TABLE 8.—*Effect of repeated forcing of fresh skim milk through the viscometer capillary*

Sample No.	Pressure (gm. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
		1	1.361
		3	1.361
		5	1.361
		9	1.361
		11	1.363
		13	1.361
1	682.9	15	1.362
		17	1.361
		19	1.363
		23	1.361
		27	1.361
		29	1.363
		35	1.363
		39	1.361
	682.1	1	1.428
	682.1	3	1.426
	681.5	5	1.428
	678.8	7	1.427
2	682.9	9	1.425
	678.8	11	1.428
	682.1	15	1.428
	682.1	19	1.428
	682.1	21	1.428
		2	1.449
		4	1.449
		6	1.449
		10	1.450
		14	1.449
3	548.3	16	1.450
		20	1.449
		24	1.449
		30	1.449
		32	1.450

In Table 8 the results obtained with skim milk are given. From these results it is apparent that continuously running skim milk through the capillary at constant pressure had absolutely no effect on the viscosity values, since they remained constant throughout each set of runs.

From the data in Table 9 it will be seen that the viscosity actually increased as the colostrum skim milk was run repeatedly through the capillary. This increase was probably due to a gradual increase in the viscosity of skim milk upon aging, for considerable time elapsed during the experiment. The higher the protein content the greater is the increase of viscosity with age. The deviation from a constant value tends to decrease as the composition approaches that of normal skim milk. The colostrum taken one day after parturition showed the greatest variation. Also it tended to stick to the walls of the viscometer reservoir, which resulted in poor drainage.

TABLE 9.—*Effect of repeated forcing of fresh colostrum skim milk through the viscometer capillary*

Days after parturition	Pressure (gm. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
1	683.4	1	3.460
	683.4	3	3.473
	683.4	5	3.485
	683.4	7	3.485
	683.4	9	3.485
	682.9	11	3.486
	682.9	13	3.497
	682.9	15	3.503
2	684.5	1	1.782
		3	1.785
		5	1.789
		7	1.789
		9	1.789
		11	1.786
		13	1.786
		17	1.787
		21	1.787
		23	1.787
3	690.9	1	1.518
	682.7	3	1.522
	682.7	5	1.522
	682.7	7	1.525
	682.7	9	1.525
	682.7	11	1.525
	682.7	13	1.525
	682.7	15	1.525

TABLE 10.—*Effect of repeated forcing of milk through the viscometer capillary*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
3	6.04	15.43	814.2	1	1.867
				3	1.847
				5	1.837
				7	1.828
				9	1.818
				11	1.816
				17	1.818
				27	1.818
				29	1.816
30	6.04	15.43	815.4	1	1.949
				3	1.915
				5	1.900
				7	1.883
				9	1.874
				11	1.868
				13	1.863
				17	1.857
				21	1.859
				25	1.857
6	3.58	12.87	814.0	1	1.640
				3	1.638
				7	1.634
				11	1.626
				15	1.626
				19	1.625
				25	1.625

The results obtained with raw whole milk are given in Table 10, and they indicate that the viscosity values decrease to a constant under the conditions of the experiment. With fresh Jersey milk the viscosity decreased from 1.867 to 1.816 centipoise. After aging for

about 30 hours, the effect of repeatedly running the same sample through the capillary was even greater, falling from 1.949 to 1.857. Similar results were obtained with Holstein milk, the viscosity dropping from 1.640 to 1.625.

A microscopic study of the whole milk which had been repeatedly run through the capillary, and that which had not been, showed that the decrease in viscosity, noted above, was correlated with a breaking up of the fat clusters. In Jersey milk especially the majority of the fat globules were initially present as clumps, but after being subjected to the above treatment the microscopic field showed very few clustered groups of fat globules. The portion of the sample which had been run back and forth through the viscometer showed very little tendency to cream after 20 minutes in the microscopic creamer, while the creaming of the control sample which was clumped was very rapid. Another source of decrease of viscosity which should be noted, was the tendency of the fat to stick to the inner walls of the viscometer reservoirs.

It has been noted that repeatedly running normal skim milk through the capillary had no effect on its viscosity, while with normal whole milk a marked decrease resulted, the effect being greater the higher the fat content of the milk. It was also found that the decrease in viscosity of whole milk was correlated with the breaking up of the aggregates of fat globules. In order to check the above conclusions homogenized whole milk was repeatedly run through the capillary at constant pressure. Representative data covering the results of these operations are included in Table 11. The viscosity values obtained with homogenized whole milk are as constant as those with normal skim milk, under the same experimental conditions. Since the fat globules in homogenzied whole milk are not clumped, this result would be expected. These experiments show that the decrease in viscosity of normal whole milk due to repeatedly running the same sample through a capillary or to agitation is accounted for by the breaking up of the clumps of fat globules.

TABLE 11.—*Effect of repeated forcing of homogenized whole milk through the viscometer capillary*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gms. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
8	3.58	12.87	814.0	1	1.830
				3	1.829
				5	1.830
				7	1.830
				11	1.829
				15	1.830
				23	1.830
6	2.94	11.63	816.7	1	1.586
			816.7	3	1.586
			816.7	5	1.586
			816.7	7	1.587
			816.7	9	1.586
			816.7	15	1.586
			816.7	19	1.586
			815.6	25	1.585
			815.6	29	1.585
			816.7	35	1.586

THE EFFECT OF HOMOGENIZATION ON THE VISCOSITY OF MILK

The influence of homogenization on the viscosity of whole and of skim milk was considered in this set of experiments. Much work has been done on the viscosity of homogenized cream, while the effect of this treatment in the case of skim milk and whole milk has received much less attention.

Buglia (*10*) demonstrated that homogenization increases the viscosity of whole milk, while it has practically no effect on skim milk. Wiegner (*32*) obtained results similar to those of Buglia for whole milk. Evenson and Ferris (*14*) also noted an increase in the viscosity of Pasteurized normal milk after it had been homogenized. Babcock (*2*) states that homogenization is very detrimental to the whipping qualities of cream, this effect being multiplied by raising the homogenization pressure. The work of Sherwood and Smallfield (*28*) is very interesting, since they studied, by means of a microscope as well as a viscometer, the effect of homogenization. They found that the rate of increase in viscosity of a sample of Pasteurized cream on aging was much greater if it had been homogenized, the increase being accompanied by a growing tendency of the globules to clump.

TABLE 12.—*Effect of homogenization on the viscosity of skim and whole milk*

Number of hours aged	Composition (percentage by weight) of—		Pressure (gms. per sq. cm.)	Viscosity (centipoise)	
	Fat	Total solids		Normal	Homogenized
6	0.11	8.87	277.3	1.383	1.362
			680.9	1.370	1.355
			980.4	1.360	1.351
8	.18	9.43	277.3	1.454	1.451
			680.4	1.432	1.422
			980.4	1.428	1.419
8	4.05	12.82	277.3	1.635	2.001
			680.1	1.623	1.998
			980.4	1.619	1.970
3	4.63	14.08	814.0	1.640	1.830
			814.0	1.640	1.831

Table 12 shows the effect of homogenization on the apparent viscosity of skim milk and whole milk. It is rather interesting to note that homogenization slightly decreases the viscosity of skim milk, while it materially increases the same physical property of whole milk. In the case of skim milk, it is probable that the state of subdivision of some of the protein clots which may be present is altered, when subjected to the violent physical forces of this treatment, so as to cause a slight diminution in viscosity.

A microscopical observation of the fat globules, before and after homogenization, showed that their average size had been greatly decreased. An increase in viscosity under the above conditions is quite in accord with the results of Ödén (*23*), who studied the effect of decreasing the particle size on the viscosity. Hatschek (*16*) concluded that this augmentation was due to an adsorption film of the liquid phase around the particles, the thickness of which was independent of the particle size.

The same explanation can be used for the effect of homogenization on the viscosity of whole milk. Homogenization reduces the size of the fat globules, and at the same time increases the specific surface of the fat phase. Since the thickness of the adsorption film varies slightly if at all with the particle size, and the amount of adsorption depends upon the total surface, it can be seen that more of the skim-milk phase will be adsorbed, in the case of homogenized whole milk, and will no longer count as free liquid. This results in an increase in viscosity, due to a decrease in the volume of the dispersion medium and an increase in the volume of the dispersed phase. In other words, the augmentation of viscosity is probably due to the adsorption of the skim-milk phase by the fat globules, and consequently the homogenized whole milk becomes more viscous than ordinary milk of the same fat content. For example, Wiegner (*32*) reduced the average diameter of fat globules in milk from 2.9 μ to about 0.27 μ by the homogenization process. From viscosity measurements he calculated that in ordinary milk about 2 per cent of the casein is adsorbed by the fat, while the casein adsorbed in homogenized milk is about 25 per cent. This amount of protein adsorbed is probably too high due to his assumption that the adsorbed protein film had a density of about 1.4.

It was also found that when homogenized milk is aged at low temperatures for several days the viscosity rises. This is undoubtedly due to the altering of the proteins.

EFFECT OF AGING ON THE VISCOSITY OF SKIM MILK

When milk stands in contact with the atmosphere, at least a slight exchange of gases occurs. Adsorption of oxygen and nitrogen and a loss of dissolved carbon dioxide takes place. Probably there is a slow change in the solubility and equilibrium of the salts. Fat globules clump and rise, resulting in a cream layer at the surface. The proteins may be slowly denaturized. The milk contains enzymes, some of which are inherent and other of bacterial origin. These enzymes alter the composition of the milk with aging. It is apparent that the study of the influence of aging on the physical properties of milk is important, since much of the market milk and cream is kept for some time at low temperatures before being used.

Kobler (*20*) believed that an initial increase in the viscosity of fresh whole milk occurred due to the gradual loss of air bubbles which were present, and that the later increase was due to the proteins, in which case the viscosity increased until the casein was precipitated. The loss of small air bubbles from the milk should, however, cause a decrease in viscosity as measured with a capillary viscometer. Burri and Nussbaumer (*11*) allowed a sample of whole milk to stand for 12 hours at about 20° C., and found that the viscosity increased with aging. Evenson and Ferris (*14*) demonstrated that milk which had been aged for one week at 3° C. showed an increase in viscosity over that kept for one hour at 30° C. Dahlberg and Hening (*13*) also made an extensive study of the influence of aging on the viscosity of milk and cream and their results, in general, agree with those of the other workers.

Most of the recorded work on aging has been done with whole milk and cream. Much of the variation in the viscosity of whole milk, as has been previously shown, can be ascribed to a variation

in the degree of clumping of the fat globules. A study of the effect of aging on the viscosity of skim milk would show whether or not the augmentation noted on aging whole milk is due to a variation in the clumping of fat globules or to an actual increase in the viscosity of the skim milk phase.

TABLE 13.—*Effect of aging on the viscosity of skim milk*

Number of days aged	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.
	Fat	Total solids		
0	0.19	9.18	983.9	1.500
			814.8	1.504
			682.2	1.506
			480.3	1.514
			278.1	1.522
			145.6	1.523
3			982.0	1.502
			814.8	1.504
			682.2	1.511
			480.3	1.517
			278.1	1.526
			147.0	1.531
			68.0	1.530
5			982.0	1.510
			814.8	1.513
			682.2	1.522
			480.9	1.528
			278.0	1.538
			148.1	1.538
			74.0	1.546
8			981.2	1.517
			814.8	1.525
			681.6	1.522
			483.0	1.541
			92.8	1.540

TABLE 14.—*Effect of aging on the viscosity of skim milk*

Number of days aged	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.
	Fat	Total solids		
0	0.12	9.87	981.2	1.500
			814.8	1.491
			681.6	1.506
			480.9	1.507
			278.1	1.519
			142.4	1.516
			71.3	1.517
3			982.3	1.518
			816.5	1.520
			683.2	1.525
			481.7	1.534
			278.8	1.539
			139.2	1.547
			67.3	1.554
5			981.7	1.518
			816.5	1.520
			683.2	1.525
			481.7	1.534
			278.8	1.539
			139.2	1.547
			67.3	1.554
8			983.7	1.557
			815.7	1.571
			683.3	1.568
			482.1	1.576
			278.8	1.586
			151.7	1.597

The samples of milk used in the foregoing experiments were obtained under the most sanitary conditions. Contamination was guarded against as much as possible. Sterilized bottles and pipettes were used throughout the work. The udder of the cow and the hands of the milker were thoroughly washed with dilute phenol and dried. The samples were milked directly into sterile cotton strainers which led into sterile Erlenmeyer flasks.

In the first set of experiments the effect of aging on the viscosity of skim milk was followed, and characteristic results are summarized in Tables 13 and 14 and in Figure 3.

Upon examining the data in Tables 13 and 14 and the curves in Figure 3, we note that, in general, the viscosity of skim milk increases with the progress of aging at low temperatures. Apparently this rise must be due to the changes that are taking place either in the composition or the state of subdivision of the milk proteins, or both. These changes may be brought about by bacterial and milk enzymes, or they may be due partially to the constituents of the milk not being in equilibrium.

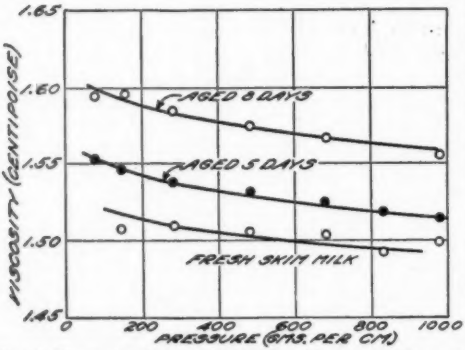

FIG. 3.—Increase in viscosity of skim milk with aging, and decrease in viscosity as shearing force is increased

In order to gain some idea as to whether or not the changes which were taking place could be attributed to acidity, determinations of the P_H and titratable acidity were made during the aging period. The results of this study are given in Table 15. In this series the milk was aged up to 25 days at about 3° C. and the effect of the storing on the viscosity, P_H, and titratable acidity were determined.

TABLE 15.—*Effect of aging at 4°–6° C. for a long period of time on some of the physical properties of skim milk* [1]

Number of days aged	Pressure (gm. per sq. cm.)	Time of flow (seconds)	Viscosity (centipoise) at 25° C.	P_H	Titratable acidity (percentage lactic acid)
1	680.8	160.7	1.433	6.78	0.14
3	680.0	164.8	1.467	6.80	.13
6	680.8	170.6	1.521	6.81	.125
9	681.6	170.8	1.525	6.83	.12
15	680.8	174.0	1.551	6.84	.11
21	681.6	174.2	1.555	6.84	.11
25	(Unable to run because of the formation of finely divided coagulum.)				

[1] Total solids=8.98 per cent; fat=0.076 per cent.

It will be seen that the apparent viscosity of skim milk gradually increased from 1.433 to 1.555 centipoise after 21 days of aging. It

was impossible to determine the "viscosity" of the milk on the twenty-fifth day because very finely divided particles were evident throughout the milk. The P_H also increased very slightly with time, and the titratable acidity lessened.

THE EFFECT OF PASTEURIZATION ON THE VISCOSITY OF SKIM MILK

This series of experiments was devoted to the study of the effect of Pasteurization on the viscosity of skim milk. Most of the Pasteurization-viscosity studies had been carried out with whole milk and cream, and the results of various workers are not all concordant. Woll (*33*), Babcock and Russell (*4*, *5*), Steiner (*29*), Evenson and Ferris (*14*), Dahlberg and Hening (*13*), and others have demonstrated that heating whole milk to Pasteurization temperatures causes a diminution of the viscosity. Achard and Stassano (*1*) and others do not agree with the majority of the workers on this subject, since they found a slight increase in the viscosity of milk when Pasteurized.

About the only work which has been done on the effect of Pasteurtization on the viscosity of skim milk is that of Whitaker, Sherman, and Sharp (*31*). They found a slight drop in viscosity when skim milk is heated to Pasteurization temperatures.

In this investigation the raw skim milk was introduced into an Erlenmeyer flask and stoppered to prevent evaporation. A thermometer was inserted through the stopper, so that the temperature of the milk could be carefully watched. The flask was placed in a water bath and the temperature was raised to about 62° C. and maintained there for 30 minutes. During this entire time the flask was continuously agitated to prevent local heating. At the end of 30 minutes it was plunged into ice water and agitated until the milk was cooled to about 4° C.

The results are summarized in Table 16.

TABLE 16.—*Effect of Pasteurization on the viscosity of skim milk* [1]

Number of days aged	Raw milk		Pasteurized milk	
	Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.	Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.
0	982.2	1.413	982.3	1.404
	682.4	1.423	683.2	1.410
	278.1	1.432	278.7	1.430
	117.9	1.439	118.0	1.429
4	982.2	1.436	981.2	1.420
	682.4	1.441	682.4	1.439
	277.1	1.452	278.1	1.446
	118.96	1.461	118.14	1.450

[1] Total solids=9.10 per cent; fat=0.15 per cent.

Table 16 brings out two important points; first, that Pasteurization slightly decreases the viscosity of skim milk. This agrees with the results of Whitaker, Sherman, and Sharp (*31*). Second, the average increase of the viscosity of Pasteurized milk due to aging, as found in these experiments, is almost the same as that of the raw milk. The process of Pasteurization should greatly reduce the number of organisms. In addition the organisms show very little action at a tempera-

ture of 3° to 4°C. These facts would indicate that the augmentation noted, which is practically the same in the Pasteurized and non-Pasteurized milk, is largely due to the altering of the proteins either by slow denaturization, by a shift of the equilibrium, by enzyme action, or by a combination of these factors.

THE EFFECT OF FREEZING ON THE VISCOSITY OF SKIM MILK

In the previous study it was noted that the viscosity of skim milk was slightly increased by aging at low temperatures. The next point considered was whether or not the viscosity of skim milk was affected by repeated freezing and thawing, and by aging in the frozen state.

The samples of milk used in this series of experiments were prepared in the same manner as those in the previous parts. Each sample was divided into several portions which were poured into sterile stoppered bottles. In every case one bottle of the milk was kept in ice water until the initial run was made, while the others were taken into a cold storage room where a temperature of −14° C. was maintained. Before making a viscosity determination the frozen sample was thawed out by immersing the bottle in lukewarm water. All samples were filtered before they were introduced into the viscometer. Filtration was necessary because of the presence of a small amount of finely divided precipitate in the frozen samples which would plug the viscometer.

The effect of repeatedly freezing and thawing, and of aging in the frozen state, on the viscosity of skim milk is summarized in Tables 17 and 18.

TABLE 17.—*Influence of aging on the viscosity of skim milk in the frozen state* [1]

Sample	Pressure (gm. per sq. cm.)	Number of days aged	Viscosity (centipoise) at 25° C.
Raw sample 1	980.6	None.	1.505
	680.2	None.	1.513
	980.6	1	1.412
	680.2	1	1.420
	980.6	33	1.472
	680.2	33	1.475
Raw sample 2	980.6	None.	1.475
	680.9	None.	1.479
	980.6	31	1.389
	680.9	31	1.391
Pasteurized sample	979.9	None.	1.462
	679.5	None.	1.468
	979.9	1	1.446
	679.5	1	1.447
	979.9	20	1.461
	679.5	20	1.470

[1] Raw sample 1, total solids=9.63 per cent, fat=0.17 per cent; raw sample 2, total solids=9.42 per cent, fat=0.08 per cent; Pasteurized sample, total solids=9.06 per cent, fat=0.096 per cent; aged at −14° C.

In spite of the fact that all of the samples of raw skim milk were subjected to almost the same treatment before and after freezing, and were aged under the same conditions, the results do not appear to be as consistent as would be desirable. A slight precipitate was always noted in the frozen samples. It was necessary to filter the frozen milk before making the viscosity determination in order to remove this precipitate so that it would not plug the capillary. Since one freezing caused the formation of a small amount of precipitate and a

decrease in the viscosity as shown in Table 17 it seemed reasonable to suppose that repeated freezing and thawing would produce increasing amounts of precipitate. This apparently was not the case, however, as shown in Table 18. Part of the milk was repeatedly frozen and thawed once each day for a week. At the end of a week this sample had about the same viscosity as another aliquot which was kept frozen the entire time. In all of the cases where a slight viscosity drop was noted, it always occurred when the skim milk was frozen the first time, and a small amount of finely divided coagulum was formed. It is interesting to note that the viscosity of the frozen skim milk dropped with the first freezing, but when aged for some time in the frozen state at −14° C., it increased until the normal value of the milk before freezing was almost reached.

TABLE 18.—*Influence of repeated freezing and thawing on skim milk* [1]

Pressure (gm. per sq. cm.)	Number of days aged	Number of times frozen	Viscosity (centipoise) at 25° C.
980.6	None.	None.	1.493
812.6			1.496
277.5			1.507
980.6	1	1	1.397
812.6			1.400
277.5			1.413
980.6	7	1	1.515
812.6			1.518
277.5			
980.6	7	7	1.514
812.6			1.515
277.5			1.513

[1] Total solids=9.65 per cent; fat=0.10 per cent; aged at −14° C.

EFFECT OF DILUTION ON THE VISCOSITY OF SKIM MILK

Many workers in searching for a shorter and a more convenient method for the determination of total solids and also for the detection of possible cases of watering, have attempted to establish a straight line relationship between the viscosity of whole milk and its composition. In the previous parts of this paper, the way in which the viscosity can vary depending on the previous treatment of the milk has been noted. This variation is especially marked in the case of the effect of the fat on the viscosity. The general application of the various formulas proposed assumes a constancy of the effect of the various constituents of milk on its viscosity. Such an assumption is unwarranted except to a very limited extent. It should be possible to eliminate one of the variable factors by the removal of the fat, but even under such conditions any relationship between the viscosity and the composition would apply only to milk samples which had the same previous treatment, and such a relationship would therefore not be general in its application.

Babcock (*3*) developed a very empirical formula which was supposed to express the relationship between the viscosity and the total solids. Babcock and Russell (*4*) later limited the use of this method by showing that the effect of fat was not constant but varied with the state of aggregation. Both Bogdan (*9*) and Oertel (*24*) concluded that the viscosity was closely related to the composition of the milk. However, the inner resistance to flow was especially sensitive to the fat

content. Taylor (*30*) believed that the total solids not fat could be calculated, if one knew the viscosity and the percentage of fat, by means of a formula which was based on the assumption that fat has a constant effect on the viscosity and varies only with the quantity. Babcock (*3*) showed that this relation did not hold, since the influence of a given amount of fat on the viscosity of milk is variable. Evenson and Ferris (*14*) compared Pasteurized normal milk with remade milk by means of the viscosity method. They assumed that a linear relation existed between the difference in the viscosity of milk and of water, and the total solids. Kooper (*21*) attempted to show that viscosity was a linear function of the total solids content, and could be used to detect cases where milk had been diluted with water. He believed that if one knew the viscosity of a diluted milk, the percentage of added water could be calculated by means of a constant (0.1384) which he obtained by dividing the average viscosity (1.588) by the average total solids content (11.472) of 50 samples of milk. This so-called constant is based on a rather illogical assumption, namely, that at zero concentration (water) the viscosity would also be zero. On the other hand, when he used this constant to calculate the added water, he subtracted the viscosity of water from the viscosity of the milk.

The effect of aging, Pasteurization, and the state of aggregation of the fat particles on the viscosity values has already been pointed out. Samples of milk might contain the same amount of milk solids not fat, and the composition of the milk solids not fat still shows enough variation to affect the viscosity.

In this set of experiments the viscosity of skim milk, systematically watered, was considered.

The skim milk was prepared as previously described, and was diluted with freshly distilled water. The water was always run into the milk from a burette until the desired dilution was made. The diluted samples were next stirred and left for two hours to allow the constituents to come to equilibrium.

The results of this study are summarized in Tables 19 and 20, and are graphically shown in Figure 4.

TABLE 19.—*Effect of dilution on the viscosity of fresh Guernsey skim milk from morning milking* [1]

Dilution water and milk (by volume)		Milk solids not fat (percentage by weight)	Time of flow (seconds)	Viscosity (centipoise) at 25° C.
0	100	9.34	163.0	1.457
10	90	8.41	154.9	1.381
20	80	7.47	147.8	1.319
30	70	6.54	141.4	1.258
50	50	4.77	128.8	1.143
80	20	1.87	112.2	0.993
100	0	0.00	----------	0.894

[1] Total solids=9.46 per cent; fat=0.12 per cent; aged 6–8 hours at 4° C.; pressure=681.9 grams per square centimeter.

TABLE 20.—*Effect of dilution on the viscosity of fresh Guernsey skim milk from morning milking* [1]

Dilution water and milk (by volume)		Milk solids not fat (percentage by weight)	Time of flow (seconds)	Viscosity (centipoise) at 25° C.
0	100	9.27	161.8	1.440
10	90	8.34	153.4	1.365
20	80	7.42	146.8	1.305
30	70	6.49	140.1	1.245
50	50	4.63	127.4	1.130
80	20	1.85	110.8	0.980
100	0	0.00	----------	0.894

[1] Total solids=9.40 per cent; fat=0.13 per cent; aged 6–8 hours at 4° C.; pressure=680.2 grams per square centimeter.

The data plotted in Figure 4 indicate that the relation between the viscosity and the milk solids not fat is expressed by a line slightly curved. However, for practical purposes where slight dilutions are being considered, a straight line relationship between the apparent viscosity of skim milk and its composition can be assumed.

A 10 per cent dilution resulted in lowering the viscosity of skim milk from 1.457 to 1.381 centipoise in one case, and from 1.440 to 1.365 in another. This means that the former was decreased by 0.076 centipoise and the latter by 0.075 centipoise by the same dilution. In a previous part a rise in viscosity of skim milk almost as great as the decrease noted above was observed, by simply allowing the milk to remain at a low temperature for several days. Since fresh skim-milk samples have occasionally been found to have a viscosity from 1.324 to 1.592 the viscosity method alone would not be reliable for detecting small amounts of added water.

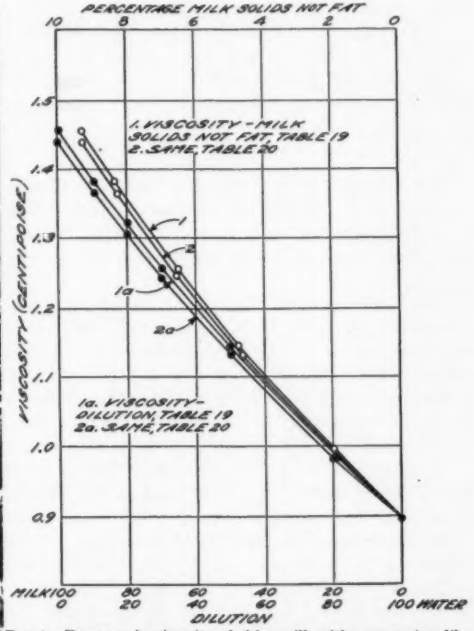

FIG. 4.—Decrease in viscosity of skim milk with progressive dilution with distilled water compared with the percentage of skim milk in the mixture and with the total solids content

DISCUSSION

The experiments reported here have shown that even the viscosity coefficient of skim milk is dependent on the shearing force used in determining it. The percentage variation in the viscosity coefficient

of normal skim milk through the range of shearing force used was small, and this slight variation could therefore be disregarded for many purposes; but the fact that such a variation does exist should not be lost sight of. The viscosity of milk can not be accurately expressed as a single value without at the same time giving the shearing force under which it was obtained.

In order to express the pressure in terms of the shearing force which is independent of the size and length of the capillary, the following relationship between shearing force and pressure was used.

$$F=\frac{rgP}{2l} \tag{8}$$

where F is the shearing force, and r and l are the radius and length of the capillary, P the pressure in grams per square centimeter, and g the gravitational constant.

The radius (0.0108 cm.) of the capillary was obtained approximately by substituting the value for C in equation (3).

Substituting the values in equation (8), the following relation between shearing force and pressure is given:

$$F=0.530\ P \tag{9}$$

The shearing force used in this work as calculated by equation (9) varied from about 36 to 780 dynes. The curves (see fig. 2) bent more sharply at the lower shearing pressures, while at the higher pressures the viscosity coefficient approached more nearly a constant limiting value.

The viscometers which are frequently used for determining the viscosity of milk, such as the ordinary Ostwald viscometer, probably operate at shearing forces between 25 and 100 dynes. They thus operate on that part of the curve where the viscosity coefficient is changing most rapidly with shearing force. Viscometers of this type must be constructed very nearly alike in order that they may give the same viscosity coefficient with the same sample of milk.

The decrease in the viscosity coefficient with increasing shearing force is not due to the breaking down of a structure in the skim milk; otherwise repeatedly running the same sample through the capillary would accomplish the same purpose. Also the increase in the viscosity of skim milk by aging can hardly be explained on the basis of a structure formed, for if this were the case one would expect that the structure would be broken down again by repeatedly running the aged sample through the capillary. The experiment with colostrum skim milk showed that the viscosity actually increased while the milk was being run repeatedly through the capillary.

Homogenization of milk and cream shows important viscosity effects; the fat globules are greatly reduced in size in both; the fat globules in the homogenized cream are markedly clumped, but clumps are practically absent in homogenized milk. This is the reason why homogenized milk does not cream to any appreciable extent, and explains why the viscosity values are so uniform with homogenized whole milk as compared with raw whole milk.

Repeatedly running normal skim milk and homogenized whole milk through the capillary of the viscometer at shearing forces from 360 to 550 dynes had no effect on the viscosity coefficient. Raw

whole milk subjected to the same treatment decreased in viscosity during the first 10 times through the capillary and after that up to 25 times through the capillary practically no further decrease in viscosity occurred. Microscopic observations indicated that this decrease in viscosity was due to the breaking up of the clumps of fat globules. Experiments in another connection have shown that as the temperature is raised the fat globules are held in the clumps less tenaciously so that it is highly probable that the decrease in viscosity due to running raw whole milk repeatedly through the capillary would have been much greater if the experiment had been carried out at a lower temperature. These experiments indicate that mechanical agitation in which air is not incorporated in the milk would have no effect on the viscosity of skim milk or homogenized whole milk but would tend to decrease the viscosity of milk in which clumps of fat globules are present.

The experiments on the effect of freezing on the viscosity were not very extensive, but they probably give an indication of the general influence of freezing on skim milk.

The decrease in the viscosity of skim milk due to dilution with water was shown to be represented by a slightly curved line. Attention should be called to the fact that as milk is diluted with water the hydrogen ion concentration decreases, as shown by Sharp and McInerney (*27*). This change in hydrogen ion concentration might produce a change in the hydration of the proteins so that until further experiments are carried out one is not justified in using this data in studying the relation between viscosity and concentration under the same conditions.

There is little doubt that taking a large number of samples, a relation exists between the total solids and the viscosity of milk, but because of the large probable error and the many factors which effect it, such as aging, Pasteurization, clumping of the fat globules, variation in the composition of the total solids, etc., the use of the viscosity as a substitute for the total solids determination of individual samples of milk is of limited value. Because of these same variables and due to the possibility of a wide variation in the viscosity of normal milk, the viscosity determination alone can not be used as a test for small amounts of added water.

CONCLUSIONS

The viscosity coefficient of whole milk, condensed skim milk, and even skim milk is not independent of the rate of shear, but decreases as the rate of shear is increased, approaching a constant value at high rates of shear.

The bending of the viscosity pressure curve is greatest in the region of shearing force which is commonly used for determining the viscosity of milk and milk products; therefore different investigators determining the viscosity of the same sample of milk would hardly obtain exactly the same viscosity coefficient, unless the viscometers which they used were nearly identical.

Mechanical agitation may cause a decrease in the viscosity of milk containing clumps of fat globules due to the breaking up of these clumps. Mechanical agitation produces no decrease in the viscosity of skim milk or of homogenized milk.

Skim milk progressively increased in viscosity with age. The aged skim milk could not be brought back to the viscosity value which it had when fresh by repeatedly running the aged sample through the capillary.

Homogenization caused a distinct rise in the viscosity of whole milk, while it caused practically no change in the viscosity of skim milk.

Pasteurization of skim milk at 62° C. for 30 minutes caused a slight decrease in viscosity when determined at 25° C.

Frozen skim milk held one day showed a decreased viscosity, but after it had aged in the frozen state for several days the viscosity increased to near that for the fresh sample.

Repeatedly freezing and thawing skim milk once each day for seven days produced the same effect on the viscosity as holding aliquot for the same length of time in the frozen condition.

The viscosity values obtained after diluting skim milk with various amounts of water fell on a slight curve, showing that the viscosity is not strictly a linear function of the total solids.

These experiments indicate that the viscosity of a sample of milk does not necessarily indicate accurately the total solids content except possibly for very restricted groups of samples.

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